

1,432,607

39840

6921

# JOURNAL OF AGRICULTURAL RESEARCH

VOLUME I

OCTOBER, 1913—MARCH, 1914

DEPARTMENT OF AGRICULTURE

WASHINGTON, D. C.

28736°—14—8

Published by Authority of the Secretary of Agriculture

---

EDITORIAL COMMITTEE

Karl F. Kellerman, Chairman  
Edwin W. Allen  
Charles L. Marlatt



## CONTENTS

	Page
Foreword. B. T. GALLOWAY.....	i
Citrus Ichangensis, a Promising, Hardy, New Species from Southwestern China and Assam. WALTER T. SWINGLE.....	1
Cysticercus Ovis, the Cause of Tapeworm Cysts in Mutton. B. H. RANSOM.....	15
The Serpentine Leaf-Miner. F. M. WEBSTER and T. H. PARKS..	59
The Occurrence of a Cotton Boll Weevil in Arizona. W. DWIGHT PIERCE.....	89
The Diagnosis of Dourine by Complement Fixation. JOHN R. MOHLER, ADOLPH EICHHORN, and JOHN M. BUCK.....	99
Three Undescribed Heart-Rots of Hardwood Trees, Especially of Oak. W. H. LONG.....	109
Individual Variation in the Alkaloidal Content of Belladonna Plants. ARTHUR F. SIEVERS.....	129
The Pubescent-Fruited Species of Prunus of the Southwestern States. SILAS C. MASON.....	147
Selective Adsorption by Soils. E. G. PARKER.....	179
A Bacterium Causing a Disease of Sugar-Beet and Nasturtium Leaves. NELLIE A. BROWN and CLARA O. JAMIESON.....	189
The Calliephialtes Parasite of the Codling Moth. R. A. CUSHMAN.	211
Polyporus Dryadeus, a Root Parasite on the Oak. W. H. LONG..	239
The Foot-Rot of the Sweet Potato. L. L. HARTER.....	251
Environmental Influences on the Physical and Chemical Charac- teristics of Wheat. J. A. LE CLERC and P. A. YODER.....	275
A Drought-Resisting Adaptation in Seedlings of Hopi Maize. G. N. COLLINS.....	293
Some Diseases of Pecans. FREDERICK V. RAND.....	303
A Twig Blight of Quercus Prinus and Related Species. DELLA E. INGRAM.....	339
New Potato Weevils from Andean South America. W. DWIGHT PIERCE.....	347
An Undescribed Species of Gymnosporangium from Japan. W. H. LONG.....	353
The Presence of Some Benzene Derivatives in Soils. EDMUND C. SHOREY.....	357

	Page
Indicator Significance of Vegetation in Tooele Valley, Utah. T. H. KEARNEY, L. J. BRIGGS, H. L. SHANTZ, J. W. McLANE, and R. L. PIEMEISEL. . . . .	365
Citropsis, a New Tropical African Genus Allied to Citrus. WALTER T. SWINGLE and MAUDE KELLERMAN. . . . .	419
Winter Spraying with Solutions of Nitrate of Soda. W. S. BAL- LARD and W. H. VOLCK. . . . .	437
Tyloses, a Study of Their Occurrence and Practical Significance in Some American Woods. ELOISE GERRY. . . . .	445
The Cambium Miner in River Birch. CHARLES T. GREENE. . . . .	471
A Study of Some Imperfect Fungi Isolated from Wheat, Oat, and Barley Plants. EDWARD C. JOHNSON. . . . .	475
The Origin of Some of the Streptococci Found in Milk. L. A. ROGERS and ARNOLD O. DAHLBERG. . . . .	491
Crystallization of Cream of Tartar in the Fruit of Grapes, WILLIAM B. ALWOOD. . . . .	513
The Reduction of Arsenic Acid to Arsenious Acid by Thiosulphuric Acid. ROBERT M. CHAPIN. . . . .	515
Index. . . . .	519

## ERRATA

Page 2, footnote, line 1, "augustis" should read "angustis."

Page 2, footnote, line 2, "ate" should read "late."

Page 28, line 30, "Taenia ovis (Cobbold, 1869) Ransom, n. comb., 1913," should read  
"Taenia ovis (Cobbold, 1869), Ransom, 1913."

Page 98, line 5, "Figs. 3 and 7.—Side and dorsal views of female" should read "Figs.  
3 and 7.—Side and dorsal views of male. Figs. 4 and 8.—Side and dorsal views  
of female."

Page 176, line 19, "Prunus havardii W. F. Wight, n. comb." should read "Prunus  
havardii (W. F. Wight), n. comb."

Page 421, footnote, line 2, "locularis" should read "locularibus."

Page 421, footnote, line 7, "locularis" should read "loculare."

Page 425, figure 4, "gabonensis" should read "gabunensis."

# ILLUSTRATIONS

PLATE	PLATES	Page
	I. <i>Citrus ichangensis</i> Swingle: The type specimen from Hsing-shan District, Hupeh Province, China. ....	14
	II. Fig. 1.— <i>Cysticercus ovis</i> from lamb which had been fed eggs of <i>Taenia ovis</i> . Fig. 2.— <i>Cysticercus cellulosae</i> . Fig. 3.— <i>Taenia ovis</i> . Fig. 4.— <i>Taenia hydatigena</i> ( <i>T. marginata</i> ) from an imported sheep dog. Fig. 5.— <i>T. hydatigena</i> ( <i>T. marginata</i> ) from a dog which had been fed <i>Cysticercus tenuicollis</i> . ....	58
	III (colored). Figs. A and B.—Portions of muscle of sheep showing <i>Cysticercus ovis</i> (undegenerated) in situ. Fig. A.—Section of hind leg showing two "deep" cysticerci. Fig. B.—Hind leg showing three "superficial" cysticerci. Figs. C and D.—Heart and portion of diaphragm of sheep showing <i>Sarcocystis</i> nodules likely to be mistaken for degenerate cysticerci. Fig. E.—Sheep heart showing numerous small degenerate cysticerci ( <i>Cysticercus ovis</i> ). ....	58
	IV. Fig. 1.—Carcass of sheep showing a degenerate cyst of <i>Cysticercus ovis</i> at the point indicated by the penknife. Fig. 2.—Degenerate cysts of <i>Cysticercus ovis</i> in muscle of sheep; portion of carcass shown in Plate III, figs. A and B. ....	58
	V. Leaves of different species, showing the work of the serpentine leaf-miner ( <i>Agromyza pusilla</i> ). Fig. 1.—Mines in a leaf of rape. Fig. 2.—Mines in leaves of white clover. Fig. 3.—Mines in leaves of alfalfa. ....	88
	VI. Figs. 1, 2, 5, and 6.— <i>Anthonomus grandis thurberiae</i> : Type specimens. Figs. 1 and 5.—Side and dorsal views of male. Figs. 2 and 6.—Side and dorsal views of female. Figs. 3, 4, 7, and 8.— <i>Anthonomus grandis</i> . Typical specimens. Figs. 3 and 7.—Side and dorsal views of male. Figs. 4 and 8.—Side and dorsal views of female. Fig. 9.— <i>Thurberia thespesioides</i> : Section of boll, showing cell of <i>Anthonomus grandis thurberiae</i> . Fig. 10.— <i>Thurberia thespesioides</i> : Seed, showing cell of <i>Anthonomus grandis thurberiae</i> . Fig. 11.— <i>Thurberia thespesioides</i> : Boll, showing egg puncture of <i>Anthonomus grandis thurberiae</i> . .	98
	VII. Fig. 1.— <i>Polyporus pilotae</i> : A sporophore on the end of a white-oak log from Arkansas. Fig. 2.— <i>Polyporus pilotae</i> : Rot appearing in the butt of a white-oak log from Arkansas, showing the holes and white cellulose areas characteristic of this rot in a cross section of a living oak. Fig. 3.— <i>Polyporus pilotae</i> : Radial-longitudinal view of a white-oak log from Arkansas, showing the honeycomb type of the rot with the white cellulose lines and elliptical hollows. Fig. 4.— <i>Polyporus pilotae</i> : Rot occurring in a log of <i>Castanea pumila</i> from Arkansas; A, concentric layers of the rotted wood; B, white cellulose fibers. Fig. 5.— <i>Polyporus pilotae</i> : Cross section of a chestnut log from New York, showing the central circular rotted zone. Fig. 6.— <i>Polyporus pilotae</i> : Radial-longitudinal view of the rot in a chestnut log from New York, showing the white pocketed stage. ....	128

PLATE		Page
VIII.	Fig. 1.— <i>Polyporus pilotae</i> : Radial-longitudinal view of the rot in a chestnut log from New York. Fig. 2.— <i>Polyporus berkeleyi</i> : Radial-longitudinal view of the rot in white-oak timber from Arkansas, showing the string and ray form characteristic of its second stage. Fig. 3.— <i>Polyporus berkeleyi</i> : A sporophore on a white-oak root from Arkansas. Fig. 4.— <i>Polyporus frondosus</i> : A sporophore on roots of white oak from Arkansas.....	128
IX.	Fig. 1.— <i>Prunus texana</i> : Better quality of fruit. Fig. 2.— <i>Prunus texana</i> : Fruiting bush, 2 meters in diameter. Fig. 3.— <i>Prunus texana</i> : Seeds; three scraped clean of pile.....	178
X.	Fig. 1.— <i>Prunus texana</i> hybrid, hort. var. <i>Stuart</i> : Fruit and leaves. Fig. 2.— <i>Prunus texana</i> hybrid, hort. var. <i>Stuart</i> : Tree in first leaf. Fig. 3.— <i>Prunus texana</i> hybrid, hort. var. <i>Johnson</i> : Fruiting branch.....	178
XI.	Fig. 1.— <i>Prunus andersonii</i> : Plant, showing taproot. Fig. 2.— <i>Prunus andersonii</i> : Flowering branch. Fig. 3.— <i>Prunus andersonii</i> : Types of seeds.....	178
XII.	Fig. 1.— <i>Prunus andersonii</i> : Tangled thickets, the more common form. Fig. 2.— <i>Prunus andersonii</i> : Treelike specimen, 3 meters high. Fig. 3.— <i>Prunus eriogyna</i> , n. sp.: Erect, large-leaved form of plant.....	178
XIII.	Fig. 1.— <i>Prunus eriogyna</i> , n. sp.: Common form of plant. Fig. 2.— <i>Prunus eriogyna</i> , n. sp.: Variable fruits and seeds. Fig. 3.— <i>Prunus eriogyna</i> , n. sp.: Fruiting branch.....	178
XIV.	Fig. 1.— <i>Prunus eriogyna</i> , n. sp.: Seedlings. Fig. 2.— <i>Prunus fasciculata</i> : Growth in flood-swept wash.....	178
XV.	<i>Prunus minutiflora</i> : Fruiting branch.....	178
XVI.	<i>Prunus havardii</i> : Fruiting branch of the type specimen.....	178
XVII.	Fig. 1.—Sugar-beet leaves inoculated with <i>Bacterium aptatum</i> . Fig. 2.—Sugar-beet root inoculated with <i>Bacterium aptatum</i> .....	210
XVIII.	(colored). Nasturtium leaves showing bacterial leaf spots 10 days after inoculation with <i>Bacterium aptatum</i> .....	210
XIX.	Fig. 1.—Bean leaves inoculated with <i>Bacterium aptatum</i> from leaf-spot of sugar beet. Fig. 2.—Nasturtium leaves inoculated with <i>Bacterium aptatum</i> from leaf-spot of sugar beet. Fig. 3.—Bean pods inoculated with <i>Bacterium aptatum</i> from leaf-spot of sugar beet.....	210
XX.	<i>Calliephialtes</i> sp. Fig. 1.—Female. Figs. 2 and 3.—Characteristic positions assumed by the insect in oviposition. Fig. 4.—Male.....	238
XXI.	Fig. 1.— <i>Polyporus dryophilus</i> : A median-longitudinal section of a sporophore on <i>Quercus alba</i> from Arkansas, showing the granular core and the white mycelial lines in the central and rear portion. Fig. 2.— <i>Polyporus dryophilus</i> : Side view of the ungulate type of sporophore on <i>Quercus californica</i> from California. Fig. 3.— <i>Polyporus dryophilus</i> : Median-longitudinal section of the globose type of sporophore on <i>Quercus garryana</i> from California, showing the large granular core and prominent white mycelial lines. Fig. 4.— <i>Polyporus dryadeus</i> : Median-	

	Page
longitudinal view of a young sporophore on <i>Quercus texana</i> from Texas, showing the fibrous, nongranular nature of the context. Fig. 5.— <i>Polyporus fulvus</i> Fries: Median-longitudinal view of a sporophore on <i>Quercus</i> sp. from Sweden, showing the granular core characteristic of <i>P. dryophilus</i> . Fig. 6.— <i>Polyporus vulpinus</i> : Median-longitudinal view of sporophore on <i>Populus</i> sp. from Sweden, showing the granular core characteristic of <i>P. dryophilus</i> . Fig. 7.— <i>Polyporus dryophilus</i> : Front view of the applanate type of a sporophore on <i>Populus tremuloides</i> from Colorado, showing the faint zones on the pileus where the hairs have disappeared. Fig. 8.— <i>Polyporus dryophilus</i> : Median-longitudinal view of sporophore on <i>Populus tremuloides</i> from Colorado, showing the granular core originating between the sapwood and bark and extending into the center of the sporophore.....	250
<b>PLATE XXII.</b> Fig. 1.— <i>Polyporus dryophilus</i> : Radial-longitudinal view of the rot occurring in <i>Quercus</i> sp. from Europe and said to be the rot produced by <i>P. dryadeus</i> . Fig. 2.— <i>Polyporus dryadeus</i> : Cross section of a small root of <i>Quercus alba</i> from Maryland, showing the mottled appearance of the diseased wood in the middle stages of the rot. Fig. 3.— <i>Polyporus dryophilus</i> : Radial-longitudinal view of the rot appearing in <i>Quercus alba</i> from Arkansas, showing the advancing line of rot in a branch. Fig. 4.— <i>Polyporus dryadeus</i> : Upper surface of a sporophore on roots of <i>Quercus texana</i> from Texas, showing the rough tuberculate pileus. Fig. 5.— <i>Polyporus dryadeus</i> : Rot occurring in an apparently sound root of <i>Quercus alba</i> from Virginia, showing cross section of a diseased root immediately adjacent to the point of attachment of a large sporophore of <i>P. dryadeus</i> , 1 foot high and 1 foot wide. Fig. 6.— <i>Polyporus dryadeus</i> : Cross section of a diseased root of <i>Quercus alba</i> from Virginia, showing the nearly sound, living upper half of the root and the badly diseased lower half.....	250
<b>XXIII.</b> Parts of sweet-potato plants, showing the presence of pycnidia: A, On the stem just above the ground; B, on the root.....	274
<b>XXIV.</b> Portion of sweet-potato vines several feet from the hill, showing the results of a natural infection of the foot-rot fungus.....	274
<b>XXV.</b> Microscopic characters of the foot-rot fungus: A, Section through a pycnidium on the root; B, section through a pycnidium on the stem; C, hyphæ, from artificial culture; D and E, chlamydosporelike bodies found on the host and in some culture media; F, pycnosporos; G, club-shaped bodies often found in pycnidia; H, germinating pycnosporos.....	274
<b>XXVI.</b> Two sweet-potato plants in pots, demonstrating the parasitism of the foot-rot fungus: A, Inoculated; B, not inoculated.....	274
<b>XXVII.</b> Nine-day-old cultures on synthetic agar: A, The conidial stage of <i>Diaporthe batatatis</i> ; B, <i>Plenodomus destruens</i> ....	274

	Page
PLATE XXVIII. Sweet potatoes inoculated with <i>Plenodomus destruens</i> : A, Inoculated at the end; B, a section of A showing extent of rot; C, inoculated at the side; D, section of C showing the extent of rot.....	274
XXIX. Fig. 1.—A seedling of Hopi maize with mesocotyl 18 cm. long. Fig. 2.—The root system of a plant of Zuni maize dug from a field near Zuni, N. Mex., showing the well-developed, single seminal root and the comparatively feeble nodal roots.....	302
XXX. Fig. 1.—A hill of Hopi maize containing 15 plants grown under conditions of extreme drought at the base of the First Mesa near Polacca, Ariz. Fig. 2.—A plant of Hopi maize.....	302
XXXI. Fig. 1.—A field of Zuni maize near Zuni, N. Mex. Fig. 2.—A hill of Zuni maize. Fig. 3.—A hill of Hopi maize making luxuriant growth under conditions of extreme drought.....	302
XXXII. Fig. 1.—A single plant of Navajo maize grown under irrigation at Shiprock, N. Mex. Fig. 2.—The basal portion of the plant of Navajo maize shown in figure 1, with leaves and husks removed.....	302
XXXIII. Fig. 1.—Pecan nuts infected with the anthracnose fungus by spraying with a distilled water suspension of conidia, showing the appearance nine days after inoculation. A, Four check nuts, two punctured with sterile needle and two unpunctured. B, Four nuts inoculated upon the unpunctured surface of the hull. C, Four nuts inoculated after puncturing the surface of the hull with a sterile needle. Fig. 2.—Three of the infected nuts shown in figure 1 after further development of the acervuli.....	338
XXXIV. Yellow Newtown apples infected by needle puncture with conidia of the anthracnose fungus from pecan and apple, showing appearance four days after inoculation. Fig. A.—Check apples punctured by sterile needle. Fig. B.—Apples infected by needle punctures with strain 150 from the apple. Fig. C.—Apples infected with strain 123 from a diseased pecan hull. Fig. D.—Apples infected with strain 125 from a diseased pecan hull.....	338
XXXV. Yellow Newtown apples infected by needle puncture with conidia of the anthracnose fungus from pecan and apple, showing appearance four days after inoculation. Fig. A.—Check apple punctured by sterile needle. Fig. B.—Apple infected with strain 125 from the pecan nut. Fig. C.—Apple infected with strain 123 from the pecan nut. Fig. D.—Apple infected with strain 150 from the apple. Fig. E.—Apple infected with strain 146 from the pecan leaf. Fig. F.—Apple infected with strain 158, a reisolation of strain 125 after passage through the apple..	338
XXXVI. Crown-gall (caused by <i>Bacterium tumefaciens</i> Sm. and Town.) on pecan nursery trees from southern Mississippi. Fig. 1.—The soft type of gall. Fig. 2.—The hard type of gall.....	338

- PLATE XXXVII (colored). Fig. A.—A pecan leaflet infected with the brown leaf-spot fungus (*Cercospora fusca*, emend. sp.) from pure culture. Fig. B.—A pecan leaflet infected with the anthracnose fungus (*Glomerella cingulata* (Stonem.) S. and v. S.) from pure culture. Fig. C.—View of upper surface of a pecan leaflet recently infected with the nursery-blight fungus (*Phyllosticta caryae* Peck) from pure culture. Fig. D.—A pecan kernel infected with the kernel-spot fungus (*Coniothyrium caryogenum*, n. sp.) from a pure culture, showing the appearance eight days after inoculation. Fig. E.—A pecan kernel with the kernel-spot from natural infection. Fig. F.—A pecan nut infected with the anthracnose fungus from pure culture. Fig. G.—The nursery-blight fungus upon synthetic agar after two weeks. Fig. H.—The nursery-blight fungus on corn-meal agar after two weeks. Fig. I.—Views of the upper and lower surfaces of pecan leaflets, showing an advanced stage of the nursery-blight. Fig. J.—The brown leaf-spot fungus on synthetic agar after four weeks. Fig. K.—The brown leafspot fungus on corn-meal agar after four weeks ..... 338
- XXXVIII. An oak (*Quercus gambelii*) inoculated with *Diplodia longispora* at X when dormant ..... 346
- XXXIX. Injury caused by potato weevils. Fig. 1.—A section of a potato from Peru, showing the larva of *Rhigopsidius tucumanus* in its burrow. Fig. 2.—A section of a potato, showing the burrowings of *Rhigopsidius tucumanus*. . . . . 352
- XL. *Rhigopsidius tucumanus* Heller. Fig. 1.—Dorsal view. Fig. 2.—Ventral view. . . . . 352
- XLI. Figs. 1 and 2.—*Premnotrypes solani* Pierce. Fig. 1.—Dorsal view. Fig. 2.—Ventral view. Fig. 3.—*Trypopermnon latithorax* Pierce. Dorsal view. . . . . 352
- XLII (colored). Sketch map showing the distribution and relative areas of the different types of vegetation in Tooele Valley, with detail showing depressions covered with salt-flat vegetation alternating with ridges bearing greasewood-shadscale vegetation. . . . . 418
- XLIII. Fig. 1.—Salt-flat vegetation bordering Great Salt Lake with a greasewood-shadscale ridge in the foreground, a pure stand of *Salicornia utahensis* at the right and hummocks covered with *Allenrolfea occidentalis* in the background. Fig. 2.—Sagebrush association and islands of *Kochia* vegetation in the upper part of Tooele Valley. . . . . 418
- XLIV. Sagebrush (*Artemisia tridentata*). Fig. 1.—A good stand and growth, showing the typical appearance of this association where the conditions are relatively favorable. Fig. 2.—Plants showing the root habit; photographed at the edge of a deep "arroyo" where the soil had recently caved in. . . 418
- XLV. Fig. 1.—Sagebrush land which has recently been burned over, showing scattered, dead plants of *Artemisia tridentata* (no living ones), a dense growth of the annual grass *Bromus tectorum*, and scattered plants of *Gutierrezia sarothrae*. Fig. 2.—An advanced stage in succession on sagebrush land which has been under cultivation, with

		Page
	numerous young plants of <i>Artemisia tridentata</i> and a dense herbaceous covering of <i>Bromus tectorum</i> and alfalfa ( <i>Erodium cicutarium</i> ). Fig. 3.—Sagebrush reestablished on land which has been in cultivation and the original, undisturbed sagebrush vegetation.....	418
PLATE	XLVI. Fig. 1.—Line of contact between the sagebrush association and the <i>Kochia</i> association, showing the characteristically sharp demarcation of the two types. Fig. 2.—A typical view of the <i>Kochia</i> association, with plants rather far apart and very uniform in size and appearance. Fig. 3.—Plants of <i>Kochia vestita</i> , 4 or 5 inches high, and the grass <i>Poa sandbergii</i> , which is usually associated with the <i>Kochia</i> in land that is not grazed.....	418
	XLVII. Fig. 1.—Typical shadscale vegetation, consisting of a nearly pure stand of <i>Atriplex confertifolia</i> , showing much deadwood, as is usually the case, but the stand is denser than in much of the area occupied by this association. Fig. 2.—Transition area between the shadscale and the greasewood-shadscale types of vegetation. Fig. 3.—Salt grass ( <i>Distichlis spicata</i> ) covering the whole of the depression to the right with the exception of a colony of <i>Allenrolfea</i> in the middle distance.....	418
	XLVIII. Fig. 1.—Salt-flat vegetation, <i>Allenrolfea</i> community. Fig. 2.—Salt-flat vegetation, showing plants of <i>Salicornia utahensis</i> . Fig. 3.—Grass-flat vegetation, <i>Sporobolus-Chrysothamnus</i> community, showing a species of rabbit brush, associated with tussock grass.....	418
	XLIX. <i>Citropsis Schweinfurthii</i> grafted on grapefruit stock ( <i>Citrus decumana</i> ), showing vigorous growth made in 2½ years..	436
	L. Fig. 1.—Yellow Bellflower apple tree in full bloom on April 16, 1912, showing effect of spraying with a solution of nitrate of soda plus caustic potash on February 2 previous. Fig. 2.—Unsprayed check tree for comparison with figure 1.....	444
	LI. Fig. 1.—A branch from a Yellow Bellflower tree in full bloom on April 10, 1913, showing the effect of spraying with a solution of nitrate of soda plus caustic soda on February 3 previous. Fig. 2.—A branch from an unsprayed check tree for comparison with figure 1.....	444
	LII. Fig. 1.—Split radial face of a creosoted hickory block, showing tyloses in a large vessel. Fig. 2.—Tangential section of <i>Aesculus octandra</i> (yellow buckeye), showing two tyloses which have grown out of one medullary-ray parenchyma cell. Fig. 3.—Cross section of valley oak, a white oak, showing young tyloses next the bark in vessels.....	470
	LIII. Fig. 1.—Cross section of a white oak, showing fully developed tyloses in the large vessels. Fig. 2.—Radial-longitudinal view, quarter-sawed surface, of the white oak shown in figure 1, showing complete closing of the vessel. Fig. 3.—Cross section of sapwood of pignut hickory, showing fully developed tyloses. Fig. 4.—Radial view of mesquite, showing "gum" droplets and formation often stimulating tyloses.....	470



	Page
PLATE	
LIV. Cross section of cow oak, a white oak, showing normal and abnormal tyloses. Fig. 1.—Wound tyloses induced by the felling of the tree and the sudden cessation of sap flow. Fig. 2.—No tyloses; empty vessels. Fig. 3.—Young and well-developed normal tyloses.....	470
LIV. Fig. 1.—Cross section of a diffuse porous wood, yellow poplar or tulip, showing scattered tyloses. Fig. 2.—Cross section of a ring porous wood, osage orange, with vasicentric parenchyma, showing abundantly developed tyloses.....	470
LVI. Fig. 1.—Cross section of western white pine, showing ray tyloses, closed vertical resin canal in young sapwood, and nuclei visible in epithelial cells of canal which is beginning to split open. Fig. 2.—Tangential section of Norway pine, showing ray tyloses.....	470
LVII. Fig. 1.—Cross section view of shortleaf pine, showing open and partly closed vertical resin canals. Fig. 2.—Heartwood of Sitka spruce, showing closed vertical canal.....	470
LVIII. Open and closed horizontal canals in sapwood. Fig. 1.—Open canal in tamarack. Fig. 2.—Partly closed canal with distended epithelial cells in Douglas fir. Fig. 3.—Young canal which has never opened in western white pine. Fig. 4.—Open canal in red spruce surrounded by thick-walled epithelium. Fig. 5.—Partly closed canal in red spruce. Fig. 6.—Closed canal in Engelmann spruce.....	470
LIX. Fig. 1.—Log from collection of woods in the Forest-Products Laboratory—a specimen of the material used in this study. Fig. 2.—Specimens of woods showing creosote penetrance in sapwood and heartwood as affected by tyloses. Specimen A.—Red oak. Specimen B.—White oak. Specimen C.—Pignut hickory.....	470
LX. Fig. 1.—River birch with bark removed, showing larval mines of <i>Agromyza pruinosa</i> . Fig. 2.—Section through wood of river birch, showing "pith-ray flecks" produced by the work of <i>Agromyza pruinosa</i> .....	474
LXI. Fig. 1.— <i>Agromyza pruinosa</i> : Larva and details. Fig. 2.— <i>Agromyza pruinosa</i> : Pupa. Fig. 3.— <i>Agromyza pruinosa</i> : Adult male. Fig. 4.— <i>Agromyza pruinosa</i> : Abdomen of adult female, showing ovipositor. Fig. 5.— <i>Symphya agromyzae</i> : Adult.....	474
LXII. Fig. 1.—Wheat seedlings from seed inoculated with spores of <i>Helminthosporium gramineum</i> and from seed externally sterilized. Fig. 2.—Barley seedlings from seed inoculated with <i>Helminthosporium gramineum</i> and from sterilized seed. Fig. 3.—Wheat seedlings from seed inoculated with spores of <i>Fusarium culmorum</i> from oat seedlings and from seed externally sterilized. Fig. 4.—Barley seedlings from seed inoculated with spores of <i>Fusarium culmorum</i> from oat seedlings and from seed externally sterilized. Fig. 5.—Oat seedlings from seed inoculated with spores of <i>Fusarium culmorum</i> from oat seedlings and from seed externally sterilized.....	490

PLATE		Page
LXIII.	Root systems of wheat seedlings grown in 6-inch pots from seed externally sterilized and from seed inoculated with <i>Helminthosporium gramineum</i> from wheat.....	490

## TEXT FIGURES

## Citrus Ichangensis, a Promising, Hardy, New Species from Southwestern China and Assam.

FIG. 1.—	<i>Citrus ichangensis</i> , n. sp.: A, Pistil after the petals and stamens have dropped but before the style has fallen off; B, stamen as seen from one side; C, two seeds deformed by mutual pressure..	1
2.—	<i>Citrus ichangensis</i> , n. sp.: Fruit showing the very low, broad, apical papilla circumscribed by a shallow furrow.....	2
3.—	<i>Citrus ichangensis</i> , n. sp.: A, Cross section of a large fruit; B, seeds..	3
4.—	<i>Citrus ichangensis</i> : A, Calyx of dwarf wild form and pedicel with bracts; B, young fruit; C, flower bud and pedicel with bracts..	4
5.—	<i>Citrus ichangensis</i> : Flowering branch from the type specimen....	5
6.—	<i>Citrus ichangensis</i> : Flowering branch of dwarf wild form.....	6
7.—	<i>Citrus ichangensis</i> : Flowering branch from a paratype in the herbarium of the Arnold Arboretum.....	7

## Cysticercus Ovis, the Cause of Tapeworm Cysts in Mutton:

FIG. 1.—	<i>Cysticercus ovis</i> : Hooks.....	17
2.—	<i>Cysticercus ovis</i> : Head and neck.....	18
3.—	<i>Cysticercus ovipariens</i> (=C. ovis): Fragment of head.....	19
4.—	<i>Cysticercus ovipariens</i> (=C. ovis): Hooks.....	19
5.—	<i>Cysticercus ovipariens</i> (=C. ovis): Papillæ on caudal bladder....	29
6.—	Hooks of <i>Taenia ovis</i> , <i>T. hydatigena</i> , <i>T. solium</i> , <i>T. balaniceps</i> , and <i>T. krabbei</i> .....	30
7.—	Sexually mature segment of <i>Taenia ovis</i> .....	31
8.—	Sexually mature segment of <i>Taenia hydatigena</i> .....	31
9.—	Gravid segment of <i>Taenia ovis</i> .....	32
10.—	Gravid segment of <i>Taenia hydatigena</i> .....	32
11.—	Surface of caudal bladder of <i>Cysticercus ovis</i> showing papillæ..	33
12.—	Surface of caudal bladder of <i>Cysticercus tenuicollis</i> showing transverse furrows.....	33
13.—	<i>Cysticercus ovipariens</i> (=C. ovis): a, Hook; b, cyst containing cysticercus cut across.....	38

## The Serpentine Leaf-Miner:

FIG. 1.—	The serpentine leaf-miner ( <i>Agromyza pusilla</i> ): a, Adult; b, side view of head; c, side view of thorax, showing characteristic color pattern; d, dorsal view of abdomen, melanic phase; e, outline of thorax, showing location of characteristic bristles...	59
2.—	Map showing known distribution of the serpentine leaf-miner throughout the world.....	61
3.—	Map showing distribution of the serpentine leaf-miner within the United States.....	62
4.—	Alfalfa leaf with eggs of the serpentine leaf-miner in situ. a, Egg, greatly enlarged; b, same, in situ, with parenchyma of leaf partly dissected away.....	66
5.—	Larva of the serpentine leaf-miner, lateral view.....	68
6.—	Puparium of the serpentine leaf-miner, ventral view.....	68
7.—	Mouth armature of larva of the serpentine leaf-miner.....	70
8.—	Diagram showing the range in temperature throughout the year at three widely separated localities at which observations were made on the serpentine leaf-miner.....	73
9.—	<i>Diaulinus begini</i> , a parasite of the serpentine leaf-miner. At left, hind leg of <i>Diaulinus websteri</i> .....	78
10.—	Larva of <i>Diaulinus begini</i> .....	79
11.—	Pupa of <i>Diaulinus begini</i> .....	79
12.—	<i>Chrysocharis parksi</i> , a parasite of the serpentine leaf-miner. a, Middle and hind legs of <i>Chrysocharis ainsliei</i> .....	80
13.—	<i>Zagrammosoma multilineata</i> , a parasite of the serpentine leaf-miner.....	81

## The Serpentine Leaf-Miner—Continued:

FIG. 14.— <i>Pleurotropis rugosithorax</i> , a parasite of the serpentine leaf-miner.	81
15.— <i>Agromyza angulata</i> .....	84
16.—Puparium of <i>Agromyza angulata</i> , with lateral view of anal appendages at left.....	84
17.— <i>Agromyza coquilletti</i> .....	85

## The Occurrence of a Cotton Boll Weevil in Arizona:

FIG. 1.— <i>Anthonomus grandis</i> , var. <i>thurberiae</i> : Prothorax.....	91
2.— <i>Anthonomus grandis</i> Boh.: Prothorax.....	91
3.— <i>Anthonomus grandis</i> , var. <i>thurberiae</i> : Head and beak: A, Female; B, male.....	92
4.— <i>Anthonomus grandis</i> Boh.: Head and beak: A, Female; B, male.....	92
5.— <i>Anthonomus grandis</i> , var. <i>thurberiae</i> : Antenna of female.....	93
6.— <i>Anthonomus grandis</i> Boh.: Antenna of female.....	93
7.— <i>Anthonomus grandis</i> , var. <i>thurberiae</i> : A, Front leg; B, middle leg; C, hind leg.....	94
8.— <i>Anthonomus grandis</i> Boh.: A, Front leg; B, middle leg; C, hind leg.....	94
9.— <i>Anthonomus grandis</i> , var. <i>thurberiae</i> : Wing.....	95

## Individual Variation in the Alkaloidal Content of Belladonna Plants:

FIG. 1.—Diagram showing the percentage of alkaloids in the leaves of individual belladonna plants at the Arlington Experimental Farm, Va., during the seasons of 1911 and 1912.....	144
---	-----

The Pubescent-Fruited Species of *Prunus* of the Southwestern States:

FIG. 1.—Map of the southwestern part of the United States, showing the range of <i>Prunus andersonii</i> , <i>Prunus fasciculata</i> , and <i>Prunus eriogyna</i> , n. sp.....	149
2.—Map of Texas, showing the known areas and probable range of <i>Prunus minutiflora</i> and <i>Prunus texana</i> .....	151
3.— <i>Prunus texana</i> Dietr.: A, Section of calyx; B, detail of calyx lobes, showing glandular margins; C, section of calyx from flower of the horticultural variety Ramsey, <i>P. texana</i> × Wild Goose plum..	155
4.— <i>Prunus andersonii</i> Gray: A, Petal; B, section of a flower; C, calyx showing ciliate margins; D, E, dried fruit; F, G, stone.....	165
5.— <i>Prunus eriogyna</i> , n. sp.: A, Section of calyx; B, detail of portion of calyx with petals, from outside, showing glandular ciliation of lobes; C, twig showing angular habit of branching, leaves and fruit attached.....	169
6.— <i>Prunus fasciculata</i> Gray: A, Section of staminate flower, showing abortive ovary and minute hairs on interior of calyx; B, calyx cup, pistillate form, showing abortive stamens; C, detail of calyx lobe; D, fecundated ovary; E, F, G, fruits, three forms; H, I, J, seed, dorsal, ventral, and side views.....	171
7.— <i>Prunus minutiflora</i> Engelm.: A, Section of flower of pistillate form, showing well-developed pistil and abortive stamens; B, section of flower, staminate form, showing well-developed stamens and abortive pistil; C, detail of calyx lobes and petals.....	173
8.— <i>Prunus microphylla</i> Hems.: A, Section of staminate flower, showing well-developed stamens and abortive pistil; B, detail of calyx from outside; C, twigs showing leaves and fruit; D, fecundated ovary.....	176

## Selective Adsorption by Soils:

FIG. 1.—Curves showing the effect of concentration on the selective adsorption of potassium from solutions of potassium by Norfolk sandy loam and by Marshall silt loam.....	185
2.—Curves showing the effect of the presence of sodium nitrate and calcium phosphate on the selective adsorption of potassium from solutions of potassium chlorid.....	187

## A Bacterium Causing a Disease of Sugar-Beet and Nasturtium Leaves:

FIG. 1.— <i>Bacterium aptatum</i> from a 2-day beef-bouillon culture stained with carbol fuchsin.....	195
2.—Filaments of <i>Bacterium aptatum</i> taken from the condensation water from a 2-day-old agar culture; stained with carbol fuchsin: a, Segmented; b, unsegmented.....	195

<b>A Bacterium Causing a Disease of Sugar-Beet and Nasturtium Leaves—Contd.:</b>	Page
FIG. 3.—Process of cell division as seen in an 18-hour-old hanging drop culture of <i>Bacterium aptatum</i> .....	195
4.— <i>Bacterium aptatum</i> showing flagella from a 2-day-old agar culture; stained with Loeffler's flagella stain.....	196
5.—Camera-lucida drawing of a portion of a cross section of sugar-beet leaf inoculated with <i>Bacterium aptatum</i> .....	206
<b>The Calliephialtes Parasite of the Codling Moth:</b>	
FIG. 1.— <i>Calliephialtes</i> sp.: Ventral view of terminal abdominal segments, showing relative position of elements of ovipositor. <i>a</i> , Valves of sheath; <i>b</i> , lance; <i>c</i> , lancets; <i>d</i> , cerci.....	216
2.— <i>Calliephialtes</i> sp.: Lateral view of terminal abdominal segments, showing relative position of elements of ovipositor. <i>a</i> , Valves of sheath; <i>b</i> , lance; <i>c</i> , lancets; <i>d</i> , cerci.....	216
3.— <i>Calliephialtes</i> sp.: Lateral view of tips of elements of ovipositor. <i>a</i> , Sheath; <i>b</i> , lance; <i>c</i> , lancet.....	217
4.— <i>Calliephialtes</i> sp.: Ventral view of male genitalia. <i>a</i> , Sheath; <i>b</i> , penis; <i>c</i> , clasper; <i>d</i> , genital palpus; <i>e</i> , cardo.....	217
5.— <i>Calliephialtes</i> sp.: Ventral view of clasping organ of male genitalia. <i>a</i> , Basal portion; <i>b</i> , clasper; <i>c</i> , genital palpus.....	217
6.— <i>Calliephialtes</i> sp.: Egg.....	219
7.—Diagram showing relation between incubation period of eggs of <i>Calliephialtes</i> sp. and average mean temperature at Vienna, Va., 1912.....	220
8.— <i>Calliephialtes</i> sp.: Dorsal view of newly hatched larva.....	221
9.— <i>Calliephialtes</i> sp.: Ventral view of head of newly hatched larva..	221
10.— <i>Calliephialtes</i> sp.: <i>a</i> , Full-grown larva; <i>b</i> , face.....	221
11.—Diagram showing relation between temperature and larval period of males and females of <i>Calliephialtes</i> sp. in the cocoon at Vienna, Va., 1912.....	224
12.— <i>Calliephialtes</i> sp.: Prepupa of female.....	225
13.— <i>Calliephialtes</i> sp.: Beginning of exuviation of female pupa.....	226
14.— <i>Calliephialtes</i> sp.: Pupa of female and tip of abdomen of male pupa.....	226
15.—Diagram showing relation between pupal period of <i>Calliephialtes</i> sp. and temperature.....	228
<b>The Foot-Rot of the Sweet Potato:</b>	
FIG. 1.—Graphic representation of growth on rice at different temperatures.	270
<b>A Drought-Resisting Adaptation in Seedlings of Hopi Maize:</b>	
FIG. 1.—Diagram of seedling maize plant, giving terminology of parts....	294
2.—Diagram showing the average size of seedlings of Chinese, Boone County White, and Navajo maize planted at different depths..	296
<b>Some Diseases of Pecans:</b>	
FIG. 1.—Cross section of pecan leaf recently infected with the nursery-blight fungus ( <i>Phyllosticta caryae</i> Peck).....	306
2.—Horizontal section of leaf recently infected with the nursery-blight fungus.....	311
3.—Cross section of a leaf infected with the brown leaf-spot fungus..	314
4.—Diagram showing measurements in length of 200 conidia.....	318
5.—Diagram showing measurements in width of 200 conidia.....	319
6.—The anthracnose fungus upon corn-meal agar: <i>A</i> , Acervulus; <i>B</i> , conidia; <i>C</i> , ascus.....	328
7.—Diagram showing ascospore measurements of the anthracnose fungus: <i>A</i> , Length of 150 ascospores; <i>B</i> , width of 150 ascospores.	328
8.—Diagram showing conidial measurements of the anthracnose fungus: <i>A</i> , Length of 150 conidia; <i>B</i> , width of 150 conidia.....	329
<b>A Twig Blight of Quercus Prinus and Related Species:</b>	
FIG. 1.— <i>Diplodia longispora</i> : A section of a pycnidium.....	340
2.— <i>Diplodia longispora</i> : Stages in development of spore. <i>A</i> , Macro-phoma stage; <i>B</i> , Diplodia stage; <i>C</i> , Diplodia spore with two septa.....	340
3.— <i>Diplodia longispora</i> : Sclerotial bodies formed in artificial media.	343
4.— <i>Diplodia longispora</i> : A section showing grouping of pycnidia...	344

A Twig Blight of *Quercus Prinus* and Related Species—Continued:

- |  |     |
|--|-----|
| FIG. 5.— <i>Diplodia longispora</i> : Types of germination. A, B, Germ tubes from end of spore; C, germ tube from side of spore..... | 344 |
| 6.— <i>Diplodia longispora</i> : A portion of mycelium showing the coalescing of the hyphæ.....                                      | 345 |
| 7.— <i>Diplodia longispora</i> : A portion of mycelium with chlamydospore-like bodies.....   | 345 |

## New Potato Weevils from Andean South America:

- |   |     |
|---|-----|
| FIG. 1.— <i>Premnotrypes solani</i> Pierce: Lateral view of prothorax and beak. | 348 |
| 2.— <i>Premnotrypes solani</i> Pierce: Frontal view of beak.....                | 348 |
| 3.— <i>Trypopermnon latithorax</i> Pierce: Lateral view of thorax and beak.     | 349 |

## Indicator Significance of Vegetation in Tooele Valley, Utah:

- |  |     |
|--|-----|
| FIG. 1.—Curve showing the relation between the salt content (in percentages of the dry weight of the soil) and the specific electrical resistance (in ohms) of the soil when saturated with water.....   | 368 |
| 2.—Monthly distribution of precipitation at Tooele, Utah (mean for 15 years).....  | 369 |
| 3.—A representative 10-meter quadrat of the sagebrush association, showing the location of each individual of <i>Artemisia tridentata</i> and of <i>Gutierrezia sarothrae</i> , these being the only woody species present.....  | 379 |
| 4.— <i>Artemisia tridentata</i> (sagebrush): A, Detail showing the wedge-shaped, 3-toothed leaves by which this plant is easily recognized; B, a small plant growing where hardpan occurred, showing the deflection of the taproot from a vertical to a horizontal direction after reaching a depth of 5 inches..... | 381 |
| 5.—A small plant of sagebrush ( <i>Artemisia tridentata</i> ), showing the deeply penetrating taproot and good development of superficial lateral roots typical of this species.....   | 384 |
| 6.—A representative 10-meter quadrat of the <i>Kochia</i> association, showing the location of each tuft of <i>Kochia</i> and of each matlike colony of <i>Poa sandbergii</i> .....  | 390 |
| 7.— <i>Kochia vestita</i> : A, Detail showing the narrow, hairy leaves; B, a plant showing the shallow root system and the propagation by root shoots.....   | 392 |
| 8.—A representative 10-meter quadrat of the shadscale association, showing the location of each individual plant of <i>Atriplex confertifolia</i> , the only woody species present, and of <i>Opuntia</i> sp.....  | 395 |
| 9.— <i>Atriplex confertifolia</i> (shadscale): A, A typical plant, showing the thick, vertical taproot and the widespreading lateral roots; B, detail of a fruiting branch, showing the shape of the leaves and of the bracts, or scales, which envelop the fruits.....  | 398 |
| 10.— <i>Sarcobatus vermiculatus</i> (greasewood): A, Detail showing the narrow, rather fleshy leaves; B, a plant showing the excellent root development.....   | 404 |
| 11.— <i>Allenrolfea occidentalis</i> : A, Detail of a fruiting branch, showing the cylindrical, fleshy, practically leafless stems; B, a plant showing the large taproot and rather scanty lateral roots characteristic of this species.....   | 409 |
| 12.—A representative 10-meter quadrat of the <i>Allenrolfea</i> community (salt-flat association), showing the location of each individual plant of <i>Allenrolfea occidentalis</i> , the only species present.....  | 410 |
| 13.—Diagram showing the characteristic root development of the dominant species of each of the principal types of vegetation of Tooele Valley and indicating the average conditions of soil moisture and salinity of the corresponding types of land.....  | 412 |

## Citropsis, a New Tropical African Genus Allied to Citrus:

- |  |     |
|--|-----|
| FIG. 1.— <i>Citropsis Schweinfurthii</i> : A branch showing 3-foliate and 5-foliate leaves, leaflike petioles, and rachis segments; also paired and single spines in the axils of the leaves.....  | 419 |
| 2.— <i>Citropsis Schweinfurthii</i> : Young seedlings germinated in Washington, D. C., from seed from Budongo Forest, Uganda, Africa. A, Young seedling, showing the first pair of leaves, succeeded by alternate simple leaves, and finally unifoliate leaves; B and D, young seedlings, showing the first foliage leaves, which are opposite; C, a single one of the pair of first foliage leaves..... | 422 |

Citropsis, a New Tropical African Genus Allied to Citrus—Continued:	Page
3.— <i>Citropsis Preussii</i> : Flowers after petals and stamens have fallen; leaves, one trifoliate and one having the terminal leaflet borne on a winged segment of the rachis. ....	424
4.—Pistils of four species of <i>Citropsis</i> . <i>A</i> , <i>Citropsis Preussii</i> ; <i>B</i> , <i>Citropsis mirabilis</i> ; <i>C</i> , <i>Citropsis Schweinfurthii</i> ; and <i>D</i> , <i>Citropsis gabunensis</i> . ....	425
5.— <i>Citropsis Schweinfurthii</i> : Nearly mature fruit; <i>A</i> , side view, showing calyx and disk; <i>B</i> , section showing four cells with pulp vesicles and three seeds. ....	427
6.— <i>Citropsis Schweinfurthii</i> : Cluster of flowers, showing stamens arranged to form a staminal tube. ....	429
7.— <i>Citropsis Schweinfurthii</i> : A trifoliate leaf from the type specimen, showing double spines in the axils and pronounced serrations of the leaflets toward the tips. ....	430
The Origin of Some of the Streptococci Found in Milk:	
FIG. 1.—Cells of streptococci, showing variation in size and morphology. .	494
2.—Types of cells of streptococci. ....	495
3.—Curve showing the typical rate of fermentation of dextrose and glycerin. ....	496
4.—Frequency curves showing acid formation in dextrose broth. ....	502
5.—Graphic representation of the characters of cultures of streptococci from milk and from bovine feces. ....	503
6.—Graphic representation of the characters of cultures of streptococci from the mouths of cows and from infected udders. ....	504
7.—Diagram showing the fermentation reactions of two types of udder cultures of streptococci. ....	506
8.—Diagram showing a possible grouping of the milk cultures of streptococci. ....	507

## FOREWORD

*The recent advances in the theory and practice of agriculture have come almost entirely from scientific research applied to agricultural problems. Accumulated results of centuries of painstaking studies have been drawn upon, and it has become evident that further improvement in agriculture calls for continued investigation of the most accurate and thorough nature. The first recognition of the economic value of progress in these investigations as well as the initial application of theories to practical problems comes usually from specialists. Indeed, only in rare instances is the significance of the results of scientific research apparent to farmers, since newly discovered facts are seldom directly applicable to agricultural conditions.*

*The suggestive or the indirect value of reports of new work is usually of paramount economic importance; it is the purpose of the Journal of Agricultural Research, therefore, to record investigations bearing directly or indirectly upon economic conditions of agriculture. It is hoped that permanence of record of new data may be secured by sending the Journal in its entirety to special libraries and institutions which make suitable exchanges and that a liberal distribution of the reprinted papers to interested specialists may enhance the usefulness of the separate articles.*

*The first few issues will contain papers from the Department of Agriculture only. Plans, however, are now being perfected in accordance with the tentative suggestions made to the Secretary of Agriculture by the executive committee of the Association of American Agricultural Colleges and Experiment Stations so that articles prepared and submitted by investigators in the State agricultural colleges and experiment stations will eventually be included in the Journal.*

B. T. GALLOWAY,  
Assistant Secretary of Agriculture.

Washington, D. C.,  
October 1, 1913.

# JOURNAL OF AGRICULTURAL RESEARCH

## DEPARTMENT OF AGRICULTURE

VOL. I

WASHINGTON, D. C., OCTOBER 10, 1913.

NO. 1

### CITRUS ICHANGENSIS, A PROMISING, HARDY, NEW SPECIES FROM SOUTHWESTERN CHINA AND ASSAM

By WALTER T. SWINGLE,

*Physiologist in Charge of Crop Physiology and Breeding Investigations,  
Bureau of Plant Industry*

#### INTRODUCTION

A study of the wild relatives of the orange begun a few years ago in the hope of finding new material for use in hybridization or as stocks has resulted in bringing to light a number of very interesting wild species, some of them new and many of them very little known. One of the most remarkable of these is a wild *Citrus*, native to southwestern China. This species is cultivated in the vicinity of Ichang, and it bears a very large lemonlike fruit that is of sufficiently good quality to cause it to be shipped to markets several hundred miles distant. It grows wild farther to the north and at a higher altitude than any other species of *Citrus* and is undoubtedly very hardy, which makes it of great promise for use in breeding cold-resistant citrus fruits. Because of its unusually large seeds it promises to yield very vigorous seedlings and to be, in consequence, a useful stock on which to graft oranges, lemons, and other cultivated species of the genus.

Mr. Augustine Henry collected excellent material of this species around Ichang, China, from 1885 to 1888. His specimens are found in many herbaria under the name "*Citrus medica* L., var." The best specimens, however, are those collected by Mr. E. H. Wilson, first in 1900 to 1903 for Veitch & Sons, and again in 1907 for the Arnold Arboretum, this latter material comprising an abundance of flowering specimens, young fruits, and also ripe fruits in alcohol.

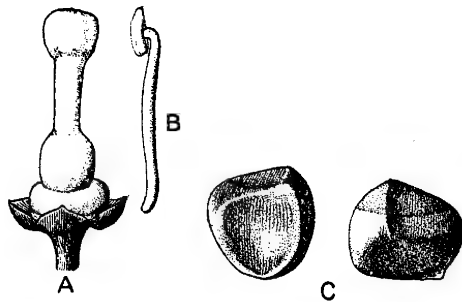


FIG. 1.—*Citrus ichangensis*, n. sp.: A, Pistil after the petals and stamens have dropped but before the style has fallen off; from a paratype in the herbarium of the Arnold Arboretum; E. H. Wilson No. 2230A;  $2\frac{1}{2}$  times natural size. B, Stamen as seen from one side; from a paratype in the herbarium of the Arnold Arboretum; E. H. Wilson No. 2230A;  $2\frac{1}{2}$  times natural size. C, Two seeds deformed by mutual pressure; from a paratype in the National Herbarium; A. Henry No. 3423 (?), bottle A; natural size. (Drawn by J. M. Shull.)



The director of the Arnold Arboretum, Prof. C. S. Sargent, has very kindly turned over to the writer all this valuable material. Thanks are also due to Mr. E. H. Wilson for furnishing very full notes about his specimens and for his observations on the use of this species as a substitute for the lemon.

In China this species occurs in an undoubted wild state in the hills of the Upper Yangtze Valley from Ichang west and southwest in Hupeh,

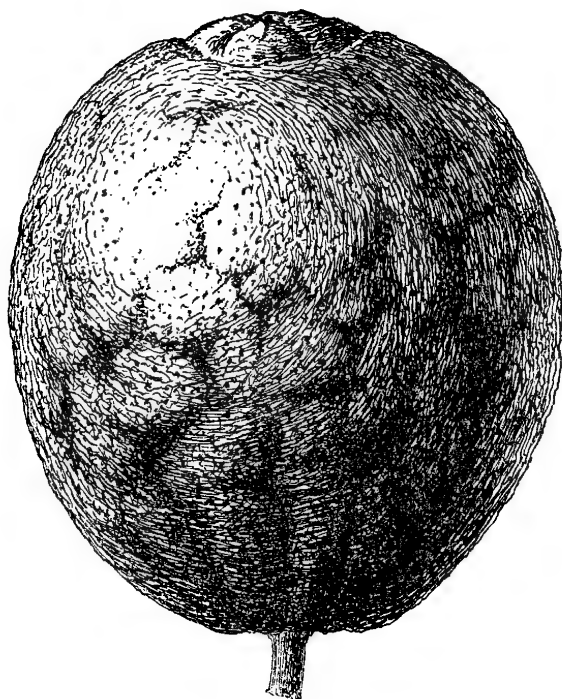


FIG. 2.—*Citrus ichangensis*, n. sp.: Fruit showing the very low, broad, apical papilla circumscribed by a shallow furrow; from a paratype in the National Herbarium; E. H. Wilson No. 4736; natural size. (Drawn by J. M. Shull.)

Szechwan, and Kweichow, growing at altitudes of 1,500 to 6,000 feet. In Assam a closely related but slightly different form is found at an altitude of 5,000 to 6,000 feet in the Khasi Hills. Doubtless other similar forms occur to the eastward in that province and in Upper Burma as well. The species thus ranges over a region at least 1,500 miles long and some 500 miles wide.

This plant is reported in all parts of its range as growing in a truly wild state and is cultivated on a small scale around Ichang along the Yangtze River, where the fruit is called the "Ichang lemon" by foreigners.

#### TECHNICAL DESCRIPTION OF CITRUS ICHANGENSIS

*Citrus ichangensis* is strikingly unlike any other *Citrus* native to China and is easily distinguished from all its congeners. Its technical description is as follows:<sup>1</sup>

<sup>1</sup> *Citrus ichangensis*, sp. nov.—*Citrus* foliis angustis, latitudine 4 plo vel 6 plo longioribus, petiolis atevalatis, obovatis vel oblongis ad basin abrupte attenuatis, laminis ovato-acuminatis, vix petiolis aequantibus, floribus grandibus, 5-meris, staminibus 20, connatis, polyadelphiis, seminibus numerosis, grandibus. Frutex vel arbor 1-10 metralis (plerumque 1-5 met.); rami juniores angulati saepe spinosisimi, 2-4 mm. diameter. Folia angusta, 60-135×15-33 mm. (plerumque 80×15-10-30 mm.), petiolis late alatis, laminis saepe aequantibus vel superantibus, obovatis ellipticis vel oblongo-spathulatis ad basin abrupte attenuatis, apice regulariter rotundatis vel truncatis vel subcordatis; laminis ovato-acuminatis plus minusve caudatis apice leviter emarginatis, ad basin regulariter rotundatis vel obtuso-cuneatis. Flores grandes, 20-35 mm. diam., 5-meri, solitarii, axillarii; pedicellis 3-5 mm. longis, calycibus sepalis crassis subtriangularibus, 3×3 mm., margine minute ciliatis; petalis oblongis 15-20×5-8 mm., staminibus 20, connatis, usque ad apicem cohaerentibus, polyadelphiis in fasciculis 3-5, 8-10 mm. longis, stylis 3-4×1½ mm., caducis; stigmatibus 2-2½ mm. longis, 3 mm. latis ovarii paullo minoribus, ovarii 3×3 mm., 8-11-locularibus. Fructus grandis, 7-10 cm. ×9-10 cm., ovalis, ad basin tuberculato-sulcatus, apice cum papilla magna vix prominenti, sulco circulari plus minusve 25 mm. diam. circumdata, cortice crasso 7-9 mm. diam.; segmentis 8-11, pulpa vesiculari acida, seminibus grandibus 15-20×10-14×7-11 mm. ovato-acutis, polyembryonicis, 40-70 in fructu singulo.

***Citrus ichangensis* Swingle.**

A spiny shrub or small tree usually 5 to 15 feet high. Leaves narrow, 4 to 6 times longer than wide, mostly 80 to 115 by 18 to 30 mm., with very large broadly winged obovate or oblong spatulate petioles evenly rounded at the tip and narrowed abruptly at the base, usually 35 to 60 by 20 to 30 mm.; with ovate-acuminate laminae more or less caudate, emarginate at the tip and evenly rounded or bluntly pointed at the base, usually 30 to 60 by 18 to 30 mm., often not equaling the winged petiole in area. Flowers about 25 mm. in diameter, 5-merous; stamens 20, at first all connate to the tips, finally breaking up into several bundles, about 10 mm. long. Pistil about 10 mm.

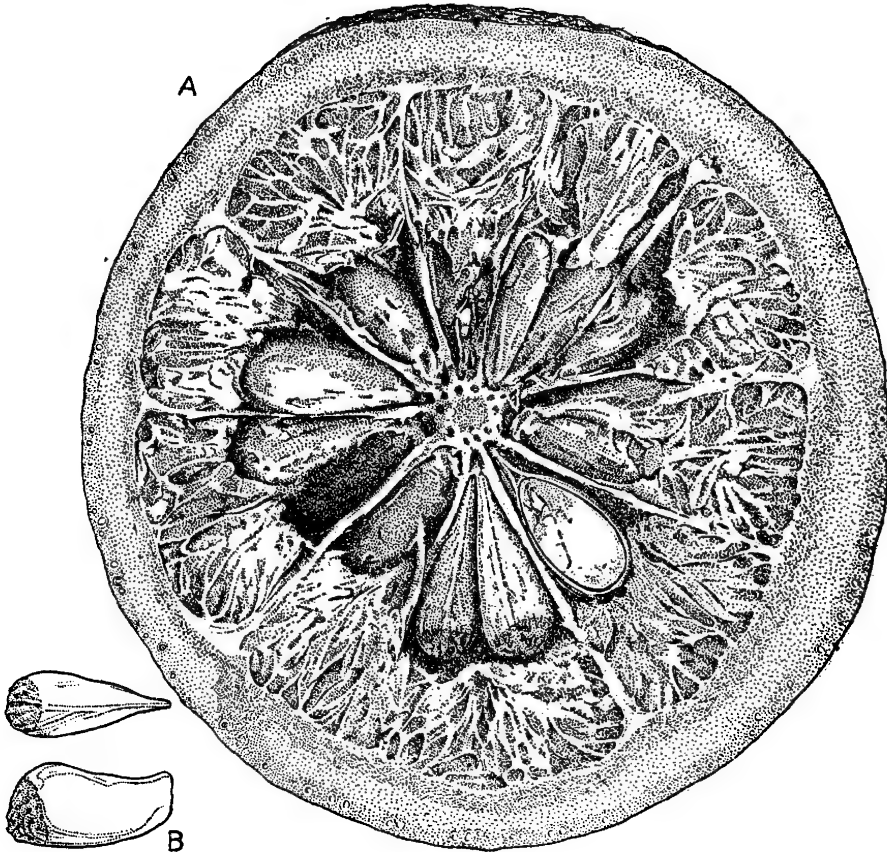


FIG. 3.—*Citrus ichangensis*, n. sp., from paratypes in the National Herbarium: E. H. Wilson No. 4737: A, Cross section of a large fruit; natural size. B, seeds; natural size. (Drawn by J. F. Brewer.)

long; stigma nearly as large as the ovary; style short, caducous; ovary 8 to 11 celled; ovules numerous in each cell. Fruit large, slightly oval, 8 to 11 by 7 to 10 cm., with a rough and furrowed base and a broad very low papilla at the tip, about 25 mm. in diameter, circumscribed by a shallow furrow; peel rather rough, 6 to 10 mm. thick. Segments 8 to 11, nearly half filled with seeds; pulp vesicles fusiform, 8 to 12 by 2 to 4 mm. on stalks 2 to 8 mm. long. Seeds very large, usually 16 to 18 by 11 to 12 by 7 to 10 mm., more or less angular from mutual pressure, 40 to 70 per fruit, polyembryonic; cotyledons thick and fleshy. (See Pl. I and figs 1 to 7.)

This species differs from its congeners in having very large thick seeds and slender leaves four to six times longer than broad, with very large, winged petioles often as large or larger than the blade. It differs from *Citrus hystrix* DC. in having oblong rather than triangular winged petioles and much larger flowers with connate stamens.

DISTRIBUTION: CENTRAL AND SOUTHWESTERN CHINA. I. HUPEH PROVINCE<sup>1</sup>

ICH'ANG PREFECTURE.—Vicinity of Ich'ang. A. HENRY, No. 3423, 1887 (?), "Thorny bush 4 ft., white flowers; in a wild jungly place; a wild plant." Flowers, Kew, Paris (Muséum), Dahlem, Harvard (Gray Herbarium), Washington,

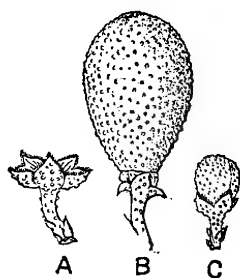


FIG. 4.—*Citrus ichangensis* from paratypes in the herbarium of the Arnold Arboretum: A, Calyx of dwarf wild form and pedicel with bracts, E. H. Wilson No. 3307, natural size; B, young fruit, E. H. Wilson No. 2230B, natural size; C, flower bud and pedicel with bracts, E. H. Wilson No. 2230A, natural size. (Drawn by Theo. Holm.)

D. C. (National Herbarium); A. HENRY, "Bottle A," "fruit from same shrub as 3423," 1887 (?); twigs and fruits, Kew; seeds (fig. 1, C), Washington, D. C. (National Herbarium). **Pingshan Pa** (in Ich'ang George, 10 km. [6¼ miles] northwest of Ich'ang), E. H. WILSON, No. 4736 (small fruit, see fig. 2), No. 4737 (large fruit, fig. 3), November, 1907, fruits only (in spirits) from cultivated trees. Harvard (Arnold Arboretum), Washington, D. C. (National Herbarium). **Ch'angyang** (25 km. [15½ miles] south-southwest of Ich'ang), A. HENRY, No. 7695, no date. "Shrub 6 to 7 ft.," fruits, Kew, sterile twigs, Harvard (Gray Herbarium); **Nanto** (20 km. [12½ miles] northwest of Ich'ang), E. H. WILSON, No. 202, April 25, 1900, flowers, Kew, Dahlem, Harvard (Arnold Arboretum), New York (Botanical Garden). **San-Yu-Tung Glen**, 10 li [4 miles] from entrance (13 km. [80½ miles] northwest of Ich'ang), E. H. WILSON, No. 2230B, July, 1907, "bushy tree, 15 ft., cultivated." Fruits (see fig. 4, B), Harvard (Arnold Arboretum). Also eight duplicate specimens for distribution. **Hsingshan District** (about 17 km. [10½ miles] southeast of Hsingshan), 10 li (5.8 km. or 4 miles) below "Liang-Shan-Kou" (altitude 1,500 to 2,000 ft.), E. H. WILSON, No. 2230, May 7, 1907,<sup>2</sup> "bush, 3 to 5 ft., flowers white, ravine," flowers, Harvard (Arnold Arboretum)

2 sheets.<sup>3</sup> (Also 8 duplicate specimens for distribution.) **Hsingshan District**, about 14 km. north-northwest of Hsingshan, 8 li (4½ km. or 3½ miles) beyond "Li-Er-Kou" (altitude 4,200 ft.), E. H. WILSON, No. 2230A, May 15, 1907, "Citrus, bush or tree, 3 to 20 ft., flowers white, escaped from cultivation, roadside," flowers (see figs. 1, A and B, and 4, C), Harvard (Arnold Arboretum). Five duplicate specimens for distribution.

<sup>1</sup> The geographic names in China are in southern Mandarin in accordance with the spelling given in L. Richard's, 1908, Comprehensive Geography of the Chinese Empire . . . Revised and translated into English by M. Kennelly. Shanghai, p. 558-639.

<sup>2</sup> Mr. Wilson's diary for this date reads as follows: "In ravine gathered specimens of a wild citrus from bushes 3 to 5 ft. tall, growing on cliffs of hard limestone." Photographs of this ravine taken by Mr. Wilson have been distributed as Nos. 025 and 032.

<sup>3</sup> One twig with flowers on one of the sheets is the type (see Pl. I and fig. 5). The other specimens of this same number resemble the type very closely, and some of them very probably were cut from the same plant, in which case they would be merotypes.

## II. SZECHW'AN PROVINCE.

KW'EICHOW PREFECTURE.—Near **Wu Shan** (35 km. [22 miles] east of Kw'eichow), A. HENRY, No. 7130, no date, fruits, Kew, British Museum, Harvard (Gray Herbarium); **Kw'eichow Gorges: Fang Hsiang Hsia** (Wind Box Gorge), E. H. WILSON, No. 3307, May 1903, "2 to 3 ft., spontaneous," <sup>1</sup> flowers (see figs. 4, A, and 6) Kew, Harvard (Arnold Arboretum).

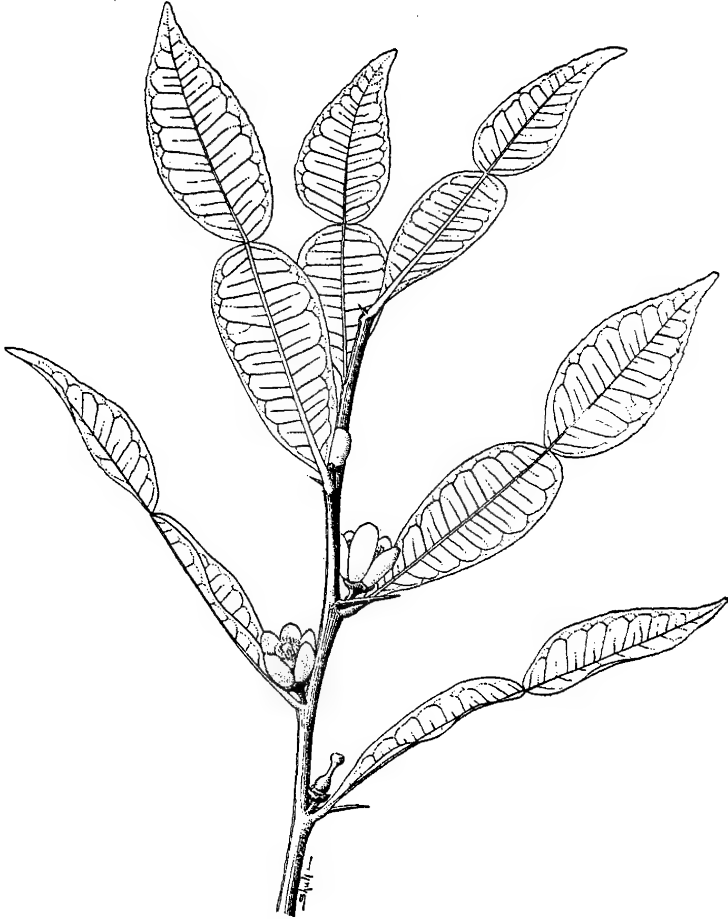


FIG. 5.—*Citrus ichangensis*: Flowering branch from the type specimen; E. H. Wilson, No. 2230;  $\frac{1}{2}$  natural size. (Drawn by J. M. Shull.)

CH'UNG K'ING PREFECTURE.—**Nanchw'an District** (about 75 km. [47 miles] south-east of Ch'ungk'ing), "Hou Ts'ao Kou,"<sup>2</sup> A. v. ROSTHORN, No. 175, July, 1891, "in dense woods," sterile twigs, Dahlem; "Huang Ai Shan,"<sup>2</sup> A. v. ROSTHORN, No. 1264, sterile twigs, Dahlem.

SUITING PREFECTURE.—**Ch'engk'ow t'ing** (about 135 km. [84 miles] northeast of Suiting), R. P. FARGES, no date, flowers, Paris (Muséum).

<sup>1</sup> Wilson, E. H., 1905, referring to this plant and locality, says: "*Citrus japonica*, 'Kumquat,' was common on the cliffs and evidently spontaneous." Gard. Chron., s. 3, v. 38, no. 969, p. 65, July 22, 1905.

<sup>2</sup> Cf. Diels, L., Die Flora von Central-China, 1900, Bot. Jahrb. [Engler], Bd. 29, Heft. 3/4, p. 424-425, Dec. 4, 1900.

## III. KWEICHOW PROVINCE.

KWEIYANG PREFECTURE.—**K'ai Chow** (?) (60 km. [37 miles] north-northeast of Kweiyang. Altitude 5,577 ft.), M. CAVALERIE, no date, young fruit, Paris (Muséum).<sup>1</sup>

## DETAILED DESCRIPTION OF CITRUS ICHANGENSIS

The typical *Citrus ichangensis* as it occurs in southwestern China is a small tree or a large shrub, usually 5 to 15 feet high (1.5 to 5 meters), but sometimes reaching 20 feet. It also occurs wild in fruiting condition only 2 to 3 feet high on the cliffs of the Yangtze Gorges.



FIG. 6.—*Citrus ichangensis*: Flowering branch of dwarf wild form; E. H. Wilson No. 2230A; natural size. (Drawn by Theo. Holm.)

The twigs of the current growth are 2 to 4 mm. in diameter and conspicuously angled, as is common in *Citrus*. The spines are straight, usually 1 to 2 cm., sometimes 2 to 3 cm. long, and 2 to 3 mm. in diameter at the base; they occur singly at one side of the axillary buds. (Pl. I and figs. 6 and 7.) Some specimens have very small spines or none at all. A few nodes at the base of the twig are often spineless.

The leaves are long and slender and remarkable because of the size of the winged petiole, which is sometimes larger than the blade. The leaves

<sup>1</sup> All of the specimens in this list have been studied by the writer and most of them have been photographed, so all are to be considered as truly paratypic.

are from 60 to 135 mm., generally 80 to 115 mm. long, and from 12 to 32 mm., mostly 18 to 30 mm. wide, the length being usually four or five



FIG. 7.—*Citrus ichangensis*: Flowering branch from a paratype in the herbarium of the Arnold Arboretum; E. H. Wilson No. 2230A; natural size. (Drawn by Theo. Holm.)

times the width. The winged petioles are obovate or spatulate oblong, rather abruptly narrowed into a wingless but sometimes margined base,

evenly rounded at the tip or sometimes truncate or subcordate, 25 to 72 by 12 to 33 mm., usually 35 to 60 by 20 to 30 mm., the wingless basal portion being 4 to 5 mm. long and  $1\frac{1}{2}$  to 2 mm. in diameter. The blades are ovate acuminate or elliptical acuminate, evenly rounded or very bluntly pointed at the base and narrowed into a more or less acuminate or caudate apex, which is, however, abruptly rounded and usually emarginate at the very tip, 20 to 66 by 13 to 30 mm., usually 30 to 60 by 18 to 30 mm. (See Pl. I and figs. 6 and 7.) The petioles and laminae have rather numerous slender secondary veins that run nearly parallel and rather straight almost to the margin, making an angle with the midrib varying from about  $45^\circ$  to nearly  $90^\circ$ . (See fig. 5.) The internodes are 12 to 30 mm., usually 15 to 20 mm., long.

The flowers are borne singly in the axils of the leaves (alongside of the spine when present); they seldom occur at the end of the twigs. The flower buds are cylindric or subcylindric, with a hemispherical tip and a truncate base, all parts being very prominently glandular dotted. (See figs. 4, 6, and 7.) The pedicels are short and slender, 4 to 6 mm. long, 1 to 2 mm. in diameter, prominently glandular dotted, with a few very small bracts near the base. The calyx is fleshy, 4 to 6 mm. in diameter; the sepals are subtriangular, 3 by 3 mm., thick and fleshy, margins minutely ciliate. The corolla is white; when fully open it is about 25 to 30 mm. in diameter, with cylindric-oval petals 12 to 18 mm. by 8 to 10 mm. wide, and 20 stamens 8 to 10 mm. long cohering almost the whole length but separating into a few bundles in fully developed flowers. The anthers are 2 to 3 by 1 to  $1\frac{1}{2}$  mm. in size. The pistil is about 10 mm. long, stout, on a cushionlike disk  $2\frac{1}{2}$  mm. high and 4 mm. in diameter, with a subglobose ovary 4 by 4 mm. The style is stout, 4 mm. long, 1 to  $1\frac{1}{2}$  mm. in diameter, caducous, leaving a very short basal portion attached to the fruit. The stigma is large, subglobose, 2 to 3 mm. in diameter, almost as large as the ovary, which is 8 to 11 celled, with numerous ovules in each cell. (See fig. 1, A.)

The fruits are subglobose, slightly longer than wide, 8 to 11 cm. ( $3\frac{1}{8}$  to  $4\frac{1}{4}$  inches) long, 7 to 10 cm. ( $2\frac{3}{4}$  to 4 inches) in diameter, with a wrinkled and furrowed base and an inconspicuous, very low, and broad papilla at the top, tipped with the persistent base of the style and delimited by a shallow circular furrow, making a circle about 20 to 35 mm. in diameter, usually 25 to 30 mm. (See fig. 2.) The fruits look like very large, short and thick lemons.

The peel is rather rough, resembling that of a large lemon, 6 to 10 mm. thick, usually 7 to 9 mm. There are from 8 to 11 segments. In a large 11-celled fruit (Wilson No. 4737) the segments are 72 mm. long, 25 to 35 mm. wide, and 20 mm. thick; in a small 8-celled fruit (Wilson No. 4736) from the same locality they are 60 mm. long, 25 mm. wide, and 18 to 22 mm. thick.

The pulp vesicles are fusiform, pointed at both ends, 8 to 12 by 2 to 4 mm., rarely reaching 18 mm. in length, on a slender stalk 2 to 8 or rarely 10 mm. long, attached to the dorsal ovary wall and also along the peripheral half of the membrane dividing the segments. The core is solid, 6 to 10 mm. in diameter, more or less stellate in cross section because of the thickening of the membranes at their attachment. The center of the core is less solid than the periphery, where there are small groups of fibro-vascular bundles opposite the attachment of each membrane.

The seeds are very large, light brown in alcoholic material, very numerous, from 40 to 70 in a single fruit and from 4 to 10 in a segment. Usually from 4 to 6 large seeds and sometimes one or more small ones occur in a segment. The seeds are cuneate ovate in outline seen from above and oval or subquadrangular seen from the side, 15 to 20 mm. long, 10 to 14 mm. wide, 7 to 11 mm. thick, mostly 16 to 18 by 11 to 12 by 7 to 10 mm., with a straight edge 6 to 8 mm. long where attached to the placenta. (See fig. 3, *A* and *B*.) They have a dark-brown cap 8 to 10 mm. in diameter at the base; the outer seed coat is thick, tough, and cartaceous, while the inner coat is thin and silky. The seeds of the wild form, collected in the vicinity of Ichang by Henry (No. 3423), are more angular through mutual pressure than those of the cultivated specimen and are also thicker. (See fig. 1, *C*.)

There are often two large embryos and usually several small ones in a single seed. Frequently the cotyledons are greatly deformed by mutual pressure of the several embryos. It is almost certain from the structure of the seeds of *Citrus ichangensis* that the cotyledons remain buried in the soil during germination, as in all the commonly cultivated species of the genus.

The dwarfed wild form of the species, found near the eastern end of the Windbox Gorge just below Kweichow (Wilson No. 3307), grows only 2 to 3 feet high and bears diminutive leaves scarcely over one-third the size of those of the cultivated form, the petioles being 16 to 23 by 7 to 8 mm. and the blades 7 to 15 by 4 to 7 mm. in size. In striking contrast to the diminutive leaves are the very numerous long spines which are unusual in showing a slight upward curvature. (See fig. 3.) Doubtless the habitat of this form on semiarid cliffs will serve to explain its small size.

Fruits collected by Augustine Henry near Ichang, likewise from a wild form, are remarkable for the fact that the numerous short, thick, and very large seeds occupy all the space in the segments, leaving room for scarcely any juice. The seeds are rather narrower in the cultivated form, but possibly this is in part due to their having an abundance of space in which to develop.

Still, in all essential characters the cultivated and wild forms agree, and doubtless the larger, juicier fruit of the cultivated form is due in part to the better nourishment the tree receives and also in part to the selection



practiced by the Chinese gardeners, who would naturally have chosen the most promising of the wild forms to propagate. Unlike many other cultivated citrous fruits, this species shows no evidence of having been hybridized; it is rather a selected form of a wild species.

Both the wild and cultivated forms of *Citrus ichangensis* will be secured as soon as possible for trial in this country. Careful exploration at higher altitudes near the northern limit of the species in China should bring to light exceptionally hardy forms that would be invaluable to breeders of hardy citrous fruits.

#### THE RELATIONSHIPS OF CITRUS ICHANGENSIS

*Citrus ichangensis* stands apart from all the other known members of the genus. Its huge, thick seeds are unlike anything heretofore known in *Citrus*, and its long, slender leaves with their very large, broadly winged petioles, often exceeding the blade in area, distinguish it at once from most of its congeners.

*Citrus hystrix* DC., a curious and little-known East Indian species, also has leaves with broadly winged petioles, often larger than the blades, but differs greatly from *Citrus ichangensis* in having very small flowers, often only 4-parted, with perfectly free stamens. Even the broadly winged petioles of *C. hystrix* are distinctly different, being more gradually narrowed toward the base and usually more abruptly truncate at the tip, making them somewhat triangular in outline, whereas those of the Chinese species are often oblong or elongate elliptical.

The other species of *Citrus* having very large, broadly winged petioles, such as *C. celebica* Koord., *C. papuana* Bail., and *C. macroptera* Montr., native to the Malayo-Polynesian region, are apparently closely related to *C. hystrix*, if, indeed, they are not to be considered as forms of it. They all agree with *C. hystrix* in having winged petioles more or less triangular in outline and show no close affinity with *Citrus ichangensis*.

The bulky seeds of *Citrus ichangensis* with their large brown caps and thick deformed cotyledons are so much larger than those of its congeners that they can not be mistaken for those of any other species of *Citrus*. They are much more like those of the African species of hard-shelled citrous fruits belonging to the genera *Balsamocitrus* and *Aeglopsis*.<sup>1</sup>

#### PREVIOUSLY PUBLISHED NOTICES OF THE SPECIES

In 1907 L. Diels<sup>2</sup> referred to *Citrus hystrix* DC., two numbers collected by A. v. Rosthorn in Szechwan in 1891, noting that one (No. 1264) had narrower leaves with inconspicuous venation and the other (No. 175)

<sup>1</sup> Stapf, Otto, 1906. *Plantae novae Daweanae in Uganda lectae*. Jour. Linn. Soc. [London] Bot., v. 37, p. 505, pl. 22.

Swingle, Walter T., 1912. Le genre *Balsamocitrus* et un nouveau genre voisin, *Aeglopsis*. Soc. Bot. France, t. 58 (s. 4, t. 11), (Mém. 8d.) p. 236 and 241, fig. B and pl. 3.

<sup>2</sup> Diels, L., 1900. Die Flora von Central-China. Bot. Jahrb. [Engler], Bd. 29, Heft. 3/4, p. 424.

broad, distinctly veined leaves. Sterile specimens of both of these numbers in the herbarium at Dahlem belong undoubtedly to *Citrus ichangensis* and differ but slightly in shape and venation.

In 1911 H. L  veill   published a "*Citrus Cavaleriei*" in an article by Julien Cavalerie<sup>1</sup> without a recognizable description. A specimen collected by P  re Julien Cavalerie in the Province of Kweichow, China, preserved in the Mus  um d'Histoire Naturelle at Paris, is almost certainly *Citrus ichangensis*. In his account of the Aurantiac   of Kweichow, he says of this species:

*Citrus Cavaleriei*, L  vl. I found in the forest, remote from any habitation in the vicinity of Ma-Jo and of Kai-Tch  ou [K'ai Chow] at about 1,700 meters [5,577 feet] altitude, a kind of spiny orange tree, in the undergrowth of the forested slopes. The tree is arched (vo  t  ) and completely covered with moss. One tree had fruits of the size of an apricot and flowers at the same time. The fruit is hard and rounded in shape; the winged petiole is so much developed that it constitutes half of the leaf. I did not see this tree cultivated anywhere. It is the only wild species [of Citrus] in the high regions.<sup>2</sup>

There is nothing in this description to distinguish this plant from *Citrus hirta* DC., and upon applying to M. L  veill   to see the type specimen he declared this name to be "a true *nomen nudum*" that had been published by mistake, and a note to this effect was later published.<sup>3</sup>

#### A SUBSPECIES FROM THE KHASI HILLS

Several good specimens of a Citrus from the Khasi Hills in Assam, collected by J. D. Hooker and T. Thomson in 1850 and preserved in the Kew Herbarium, were at first supposed by the writer to be identical with *Citrus ichangensis*, as they showed the same peculiar, very large and broadly oval or oblong winged petioles. After careful study, however, the Khasi specimens were found to differ from the typical Chinese material in a number of points.

In the first place, all of the Khasi specimens show leaves with less acuminate blades than those of the Chinese material; moreover, the leaves of the Indian specimens are distinctly more variable both in size and in shape. The immature fruits collected by Hooker and Thomson in this locality are all slightly oblate instead of slightly prolate like the Chinese fruits from Pingshan Pa (Wilson Nos. 4736, 4737). The fact that Hooker and Thomson call this plant a "wild orange" is additional evidence that the fruits did not have the lemonlike appearance of the Chinese form. Finally, the flowers in Clarke's specimen preserved in the British Museum occur in three to six flowered axillary panicles instead of singly, as in all the Chinese material seen. The tree reaches a height of

<sup>1</sup> Cavalerie, Julien, 1911. Les Aurantiac  es du Kouy-Tch  ou. Bul. de G  ogr. Bot., t. 21 (ann. 20, s. 4), no. 261, p. 211.

<sup>2</sup> Translation from Cavalerie, Julien, 1911, loc. cit.

<sup>3</sup> L  veill  , H., 1911. Les Aurantiac  es du Kouy-Tch  ou. Bul. de G  ogr. Bot., t. 21 (ann. 20, s. 4), no. 262, p. 236.

30 feet in the Khasi region and has not been recorded over 20 feet in China. This, however, might easily be due to differences in the exposure, orange trees growing in a forest often being much taller than those in the open without shade.

More material and, above all, ripe fruits will be needed to decide definitely whether the Khasi "wild orange" belongs to *Citrus ichangensis*. It is certainly much more closely related to this latter species than to any other. For the present it seems best to consider it as a subspecies of the Ichang lemon. The technical diagnosis is as follows:<sup>1</sup>

***Citrus ichangensis latipes* Swingle.**

Differs from *C. ichangensis* in having the leaves more variable in size and shape with the tips acute, not caudate, the flowers in few-flowered (3 to 5) panicles instead of solitary, and the fruits oblate instead of prolate spheroidal in shape.

DISTRIBUTION: ASSAM, NORTHEASTERN INDIA. KHASI HILLS

**Living Bridge**,<sup>2</sup> Hooker and Thomson, September 2, 1850, "small orange, *wild*," fruits, Kew; **Myrung Wood** (altitude 5,700 ft.), J. D. HOOKER and T. THOMSON, July 6, 1850, "Aurant. Tree 30 ped. alt. Frt size of a walnut," fruits, Kew; **Moflong** (altitude 6,000 ft.),<sup>3</sup> J. D. HOOKER and T. THOMSON, July, 1850, fruits, Kew; **Moflong(?)**, J. D. HOOKER and T. THOMSON, "*Citrus latipes* H. f. and T. Regio temp. (indig.) alt. 5,000-6,000 ped.,"<sup>4</sup> no date, sterile twigs, Harvard (Gray Herbarium); **Khasi Hills**, C. B. CLARKE No. 21879 (Collector Rutton), 1873, flowers, British Museum.

DETAILED DESCRIPTION OF CITRUS ICHANGENSIS LATIPES

The leaves of *Citrus ichangensis latipes* vary greatly in size and shape, ranging from 65 to 152 by 12 to 48 mm., the length varying from three to seven times the width. The petioles in particular, though always broadly winged, are distinctly more variable than in the Chinese material. They vary from oblanceolate linear to spatulate oblong or elongate obcordate. The largest petioles occur in a fruiting branch from Living Bridge (the type specimen of the subspecies in Kew Herbarium); they are spatulate oblong, 75 to 92 by 44 to 48 mm., tapering rapidly into a marginate base 4 to 6 mm. long. A specimen from Moflong (in Kew Herbarium) has oblanceolate-linear petioles 30 to 45 by 10 to 16 mm. The other material is intermediate between these two extremes, and one twig from Myrung Wood (in Kew Herbarium) has elongate-obcordate petioles 35 to 45 by 16 to 20 mm. in size. The blades of the leaves vary from ovate to lanceolate and are 35 to 65 by 14 to 40 mm.; in some specimens the laminae are decidedly smaller than the winged petiole, while in others the reverse is true.

<sup>1</sup> *Citrus ichangensis latipes*, subsp. nov.—*Citrus ichangensis* affinis, foliis acutis haud caudatis, floribus in paniculatis pauci-floribus (3-5) dispositis, fructibus oblati.

<sup>2</sup> This is the type of the subspecies.

<sup>3</sup> Cf. Hooker, J. D., 1854, *Himalayan Journals*, London, v. 2, p. 288, 292, 323.

<sup>4</sup> This specimen has only a lithographed label with manuscript additions. One of the twigs has extremely long and slender winged petioles like the specimen from Moflung in Kew Herbarium and probably was a part of the same collection. The other specimens of Hooker and Thomson in Kew Herbarium have this same label carrying in manuscript the name "*Citrus latipes* H. f. and T.," but have in addition original labels giving the exact locality and date of collection.

## PREVIOUSLY PUBLISHED NOTICES OF THE SUBSPECIES

Very little has been published concerning this plant. The first notice seems to have been given it in 1874 by Edmund Goeze, who lists it as "*Citrus latipes* Hook. fil. et Th. A very peculiar species from India."<sup>1</sup>

In 1875 J. D. Hooker, in his *Flora of British India*,<sup>2</sup> cited it under the name "*C. latipes* Hook. f. and Thoms. Herb. Ind. Or." as a synonym of *C. hystrix* DC., an erroneous determination doubtless due to the lack of flowers and mature fruits in the Khasi material at his disposal. The name "*Citrus latipes* Hook. f. and Thoms." is a *nomen nudum* without standing in taxonomy, since no description has been published under it.

Efforts are being made to secure ripe fruits and viable seeds of this interesting tree, which, like the Chinese form of the species, promises to be very cold resistant.

## POSSIBLE USES OF CITRUS ICHANGENSIS

Mr. E. H. Wilson informs the writer that the form of this species cultivated in the Ichang region yields an excellent fruit known to foreign residents of the Yangtze Valley as the "Ichang lemon." These fruits are shipped down the river to Hankow and west well into Szechwan, and are so much esteemed as to command good prices.

The large size of the seeds makes it probable that *Citrus ichangensis* will produce very vigorous seedlings, and hence it is likely to be of value as a stock on which to graft other citrous fruits. These numerous large seeds, which promise to render this plant so valuable as a stock, have the drawback of greatly reducing the proportion of juice, because of the space they take up. However, experience has shown that it is relatively a simple matter to breed nearly seedless varieties of citrous fruits by selection or hybridization.

So far as is now known, *Citrus ichangensis* is native farther north than any other evergreen species of *Citrus*, only the deciduous *Citrus trifoliata* having a more northerly range. Besides having the northernmost range of any known evergreen species of *Citrus* it occurs at the highest altitudes reported for any wild species of the genus. In the Hsingshan District, in latitude 31° 10', Mr. Wilson collected this plant at an altitude of 4,200 feet, and Père Cavalerie found it in central Kweichow at a height of 5,577 feet.

At Moflong in the Khasi Hills, Hooker and Thomson found the Khasi subspecies growing wild at an altitude of 6,000 feet. As to the winter climate of this part of Assam J. D. Hooker says:

In November the vegetation above 4,000 feet turns wintry and brown, the weather becomes chilly, and though the cold is never great, hoarfrost forms at Churra, and water freezes at Moflong.<sup>3</sup>

<sup>1</sup> Translation from Goeze, Edmund, 1874. Ein Beitrag zur Kenntniss der Orangengewächse. Hamburg, p. 19.

<sup>2</sup> Hooker, J. D., 1875. *Flora of British India*. v. 1, London, p. 575.

<sup>3</sup> Hooker, J. D., 1854. *Himalayan Journals*, v. 2, London, p. 323.

Around Ichang, which is situated at an altitude of about 2,000 feet, the winters may be severe, as is proved by the meteorological record for the year 1888, which showed an absolute minimum of 22° F. (−5.6° C.) in February.<sup>1</sup> It is highly probable that a series of observations extending over a number of years would show that the minimum temperature occasionally falls decidedly lower than this. It would undoubtedly be colder at an altitude of 4,200 feet in the near-by Hsingshan District, where this species grows wild.

Mr. Wilson, who knows the climate of this part of China well, is confident that the "Ichang lemon" will prove to be one of the hardiest citrous fruits. Add to this the fact that the fruit is of a quality good enough to cause it to be exported to cities several hundred miles distant and it is obvious that this strikingly distinct new species of *Citrus* promises to be of value as a hardy substitute for the lemon, as well as a vigorous and hardy stock for other citrous fruits, and is eminently deserving of the attention of experimenters for use in the breeding of new types of hardy citrous fruits now so much in demand in this and other countries.

Its discovery in a part of China as accessible as Ichang is a further proof of the rich harvest of new species of plants that awaits the botanist and agriculturist in China.

#### DESCRIPTION OF PLATE

PLATE I. *Citrus ichangensis* Swingle: The type specimen from Hsingshan District, Hupeh Province, China, E. H. Wilson, No. 2230, May 7, 1907; in the herbarium of Arnold Arboretum; natural size.

---

<sup>1</sup> Doberck, William, 1889. Meteorological observations made at Ichang, China, and at South Cape Formosa, in 1888. *Quart. Jour. Roy. Met. Soc. [London]*, v. 15, no. 72, p. 242.



# CYSTICERCUS OVIS, THE CAUSE OF TAPEWORM CYSTS IN MUTTON

By B. H. RANSOM,  
*Chief, Zoological Division, Bureau of Animal Industry*

## INTRODUCTION

It has been known for nearly half a century that cysticerci occur in mutton, but they have generally been looked upon as zoological curiosities rather than parasites of real economic importance; in fact, it seems that this opinion has been so commonly accepted as an established truth that a systematic examination of sheep for such cysticerci, or measles, like that given cattle and hogs, has been considered unnecessary by meat-inspection authorities. So far as this country is concerned, however, the belief that sheep measles are rare has been lately discovered to be quite erroneous. Instead of being rare, sheep measles have been found to be of much the same order of frequency as beef measles and far more common than pork measles, which are almost unknown in the United States. Where the presence of measles has been carefully looked for, the percentage of affected sheep has run 2 per cent and over, and during the calendar year 1912 approximately 20,000 sheep carcasses were retained under Federal inspection at various abattoirs on account of measles, most of them during the last few months of the year.

In the light of these figures it is quite evident that the mutton cysticercus is far from being the unimportant parasite it is commonly assumed to be, and it is furthermore quite certain that as inspectors become generally more familiar with this parasite and with the proper methods of inspecting for its presence the percentage and gross number of cases found will materially increase.

As yet sufficient data are not at hand to indicate the extent of direct injury to sheep by the measles parasite, so that the chief practical importance of sheep measles recognized at the present time is in their relation to meat inspection and public health. Like beef and pork cysticerci, the mutton cysticercus is of special interest in meat inspection because it affects the musculature, that part of the animal which is at once the most valuable for food purposes and the most difficult to inspect thoroughly.

The beef and pork cysticerci are well known to be the intermediate stages of two species of tapeworms occurring in man. The question naturally arises, Is the mutton cysticercus likewise the intermediate stage of a human tapeworm? The leading foreign meat-inspection authorities have held that the mutton cysticercus is simply *Cysticercus cellulosae*, the pork cysticercus, in an unusual host, and have laid down identical

regulations governing the disposal of affected hog and sheep carcasses. The American meat-inspection regulations, which are similar to, though necessarily somewhat more stringent than, the German regulations because of the lack of a Freibank system in this country, require the condemnation of carcasses heavily infested with *C. cellulosa* and permit slightly infested carcasses to be rendered into edible fat. As a condemned carcass is entirely destroyed for food purposes and as the value of a sheep carcass rendered into edible tallow is scarcely greater than that of one which has been condemned and made into fertilizer or other inedible products, a carcass infested with *C. cellulosa* in any degree whatsoever would be practically excluded from use as food under American regulations. Accordingly, if the mutton cysticercus were actually *C. cellulosa*, the 20,000 sheep carcasses in which muscle cysticerci were found last year would have been eliminated from the meat supply of the United States. Relatively this loss would not have been very great, and in actual money value it would not have exceeded \$100,000. In the future, however, much greater losses would occur, because the more efficient methods of inspection which would be developed by experience would naturally lead to the detection of more nearly all the cases of sheep measles than the earlier, less efficient methods. The number of sheep affected with measles is probably considerably in excess of 1 per cent of the entire number slaughtered, and accordingly the loss on this account would be very large if anywhere near all the cases were found on inspection and if they were disposed of under the assumption that the parasite involved is *C. cellulosa*.

Shortly following the discovery of the first cases found last year, the writer undertook an investigation of the question of sheep measles, with the result that it was quickly proved that the parasite involved is certainly not *Cysticercus cellulosa*, though closely resembling it in some respects, and in due course of time it was definitely established that the mutton cysticercus is the larval stage of a dog tapeworm.

The question of sheep measles is therefore much less serious than it would be if the parasite were one transmissible to man, particularly if it were the rather dangerous *Cysticercus cellulosa*. So far as meat inspection is concerned, however, sheep measles, though less important as a public-health question, are almost as important as though the parasite involved were transmissible to human beings, because meat containing parasites of sufficient size to be noticeable is more or less objectionable as food for esthetic reasons if on no other account.

#### HISTORICAL SUMMARY

Considering critically the various statements which have appeared relative to muscle cysticerci in sheep prior to the recent investigations by the present writer, it may be noted in the first place that excepting one of Morot's (1899e)<sup>1</sup> cases (No. 3), which was quite evidently one of

<sup>1</sup> Bibliographic references in parentheses refer to the "Bibliography," pp. 54-57.



generalized coenurosis, there is no definite conclusive evidence that more than one species of parasite is concerned in sheep measles; hence the presumption is that the muscle cysticerci reported from sheep all belong to a single species. Taking into account the fact that it has now been proved by experiment that muscle cysticerci in sheep develop into tapeworms distinct from either *Taenia solium* or *T. hydatigena*, it is quite clear that none of the observers reporting muscle cysticerci in sheep has given sufficient evidence to show that the parasites in any instance were *Cysticercus cellulosae*, as they were held to be by some, or *C. tenuicollis*, as they were held to be by others, and not in all cases, *C. ovis*. Commonly the only evidence to support the observer's identification is a statement that the parasite showed the characters of *C. cellulosae* (Olt, Armbrüster, Colberg, Rickmann, Herter) or *C. tenuicollis* (Chatin, Glage). In a few cases measurements of the hooks have been recorded, but these apply equally as well or better to *C. ovis* than to *C. cellulosae* or *C. tenuicollis*. Bongert's report is of special interest in this connection, as he gives a photomicrograph of the hooks (fig. 1), comparison of which with the hooks of *C. cellulosae* shows that the hooks agree imperfectly, thus



FIG. 1.—*Cysticercus ovis*: Hooks,  $\times 275$ . (After a photomicrograph by Bongert, 1899a, fig. 3.)

demonstrating the incorrectness of Bongert's positive opinion that the parasite was *C. cellulosae*. The opinion formerly held by the present writer (1908d) that certain partially grown muscle cysticerci with hooks not yet fully developed which had been found in a sheep were *C. cellulosae* on account of the presence of certain characters also found in *C. cellulosae* is likewise seen now to be quite erroneous.

Railliet and Morot noticed that the hooks of a cysticercus resembling *Cysticercus cellulosae* from a sheep heart, though agreeing fairly well in size with *C. cellulosae* hooks, as shown by the measurements which they give, corresponded closely in form to those of *C. tenuicollis*. They accordingly so identified the cysticercus, at the same time, however, calling attention to the fact that the hooks are fewer in number than is usual in *C. tenuicollis* and that they are smaller, differences possibly to be attributed, according to their view, to the location of the parasite in the muscles instead of in the serous membranes. It is quite probable—in fact, not to be doubted—that the parasite in this case was *C. ovis*.

Not only have observers failed to give sufficient evidence that the mutton cysticerci in any case exactly agreed in morphology with *Cysticercus cellulosae* or *C. tenuicollis*, but they have also failed to produce experimental proof to support their identifications. *C. cellulosae* has never been produced experimentally in sheep by feeding *Taenia solium* eggs (Leuckart, Küchenmeister, Perroncito); nor, vice versa, has *T. solium* been produced in man as a result of ingesting mutton cysticerci (Chatin, Ransom<sup>1</sup>).

There is also no good evidence that *Taenia hydatigena* has ever been obtained as a result of feeding the mutton cysticercus to dogs. It is true that Chatin states that such is the case, but the evidence that the

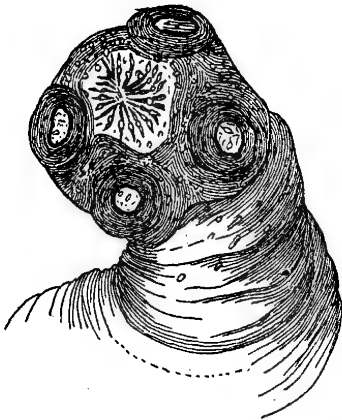


FIG. 2.—*Cysticercus ovis*: Head and neck,  $\times 30$ . (After Cobbold, 1869a, p. 30, fig. 2.)

tapeworms were identical with those belonging to *Cysticercus tenuicollis* consists simply in Chatin's affirmation that they were the same, and there is no objective evidence at all to support this view. It also should be noted that no one has shown that segments of *T. hydatigena*, when fed to sheep, will produce muscle cysticerci. Leuckart, Küchenmeister, and others have found only *C. tenuicollis* as a result of such experiments.

Cobbold's opinion that *Cysticercus ovis* is the larva of a human tapeworm, the so-called *Taenia tenella*, has never had any supporting evidence and, of course, is now entirely discredited. Cobbold, however, it is interesting to note, was quite correct in

another opinion which he at one time held—namely, that it is probable that the adult of *C. ovis* occurs in one of the carnivora.

Most of the records of muscle cysticerci in sheep are based upon isolated cases in which the parasites have usually been more or less degenerate. Thus, Cobbold noted the presence of degenerated cysticerci in mutton on several occasions and described *Cysticercus ovis* on the basis of a single specimen (fig. 2) which had lost the caudal bladder before it came into his hands. Maddox described *C. ovipariens* (figs. 3 and 4) on the basis of one degenerated cysticercus. The number of cases seen by Möbius, reported by Küchenmeister, is not stated. Chatin apparently saw muscle cysticerci on several occasions, and some of these evidently were alive and undegenerated. Morot refers specifically to five cases and refers to an indefinite number of others, in all of which the parasites were degenerated and were recognized as cysticerci only from the character of the cysts. Railliet and Morot reported one case of a single, apparently undegenerated cysticercus in the heart of a sheep, and refer

<sup>1</sup> For an account of the present writer's experiments, see pp. 21-26.

to a similar case of cysticercus in the heart of a kid. The case reported by Olt and Bongert showed numerous cysticerci, some of which apparently were alive. In another case seen by Olt the parasites were all degenerate. Armbrüster found calcified cysticerci in 2 or 3 sheep out of a shipment of 16 head. One case of muscle cysticerci was found by Colberg in which numerous degenerated parasites were present.

In a case of cysticerci in a sheep heart reported by Railliet the parasites were very young, without hooks. Glage is the only author thus far who has given a detailed statistical record of the frequency of muscle cysticerci in sheep. His records, however,

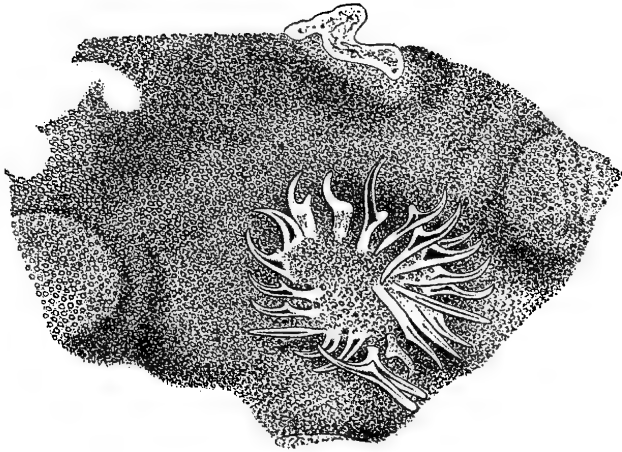


FIG. 3.—*Cysticercus ovipariens* (= *C. ovis*): Fragment of head,  $\times 85$ . (After Maddox, 1873a, pl. 19, fig. 1.)

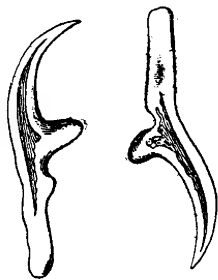


FIG. 4.—*Cysticercus ovipariens* (= *C. ovis*): Hooks,  $\times 160$ . (After Maddox, 1873a, pl. 18, fig. 5.)

are based entirely upon the presence of degenerated cysticerci, and it is not improbable that he overlooked many cases of live cysticerci. He found 32 cases (1.45 per cent) among 2,198 carcasses in which the head muscles and hearts were examined and 16 cases (0.8 per cent) among 1,984 carcasses in which only the hearts were examined. Rickmann fails to state the number of cases observed. The cysticerci in the one case reported by the present writer in 1908 were undegenerate but only partly grown. Herter mentions one case and says that only nine cases of sheep measles were recorded in the meat-inspection reports of Prussia for the year 1909. Making a very liberal allowance for the number of indefinitely reported cases, the total number of individual cases of sheep measles reported in the literature prior to the recent investigations in this country is considerably less than 100, and in only a very few of these were the cysticerci at all numerous or present in a living, fully developed, undegenerated condition. It is accordingly not surprising that the identity of these parasites should have remained so long undiscovered, particularly in view of the fact that they have received but little attention from experienced parasitologists, who, moreover, have had very unsatisfactory material for study.

Cobbold, for example, apparently studied only one specimen (imperfect), and Railliet seems to have had only one fully developed undegenerated specimen for critical examination.

Up to the present time sheep measles have been reported from the following countries: England, Germany, France, Algeria, German South-west Africa, New Zealand, and the United States.

In completing this brief critical summary of the literature, only a few words need be given concerning the morphology of the parasites. As already noted, morphological details have been omitted from most of the accounts given of the recorded cases. The measurements of the hooks given by Railliet and Morot correspond to *Cysticercus ovis*, as do Bongert's measurements and photomicrograph. Maddox was the first to observe the mammillated surface of the caudal bladder, which, however, has not been recognized as a distinctive difference between *C. ovis* and *C. tenuicollis*, except by the present writer (1908d), and apparently has escaped attention from other observers.

#### LIFE-HISTORY INVESTIGATIONS

Under date of February 29, 1912, Dr. S. E. Bennett, inspector in charge at Chicago, Ill., reported to the Bureau of Animal Industry that a number of sheep carcasses had been found to be infested with measles, and under date of March 1 Dr. O. B. Hess, inspector in charge at Seattle, Wash., also reported the finding of measles in several sheep carcasses. Specimens were forwarded to Washington from both stations for laboratory examination. The cysts in the specimens were all degenerate, but fragments of the caudal bladder of cysticerci were found, and in view of the presence of cuticular papillæ, which are likewise present on the caudal bladder of *Cysticercus cellulosæ*, and in accordance with the opinion of German meat-inspection authorities as to the identity of mutton cysticerci, the diagnosis of *C. cellulosæ* was made. Shortly following the first reports, information was received that out of 4,537 sheep slaughtered at Seattle, Wash., 79 carcasses were retained on account of measles, and that during a month at Chicago 224 carcasses were retained.

With this information at hand it was immediately apparent that the diagnosis of *Cysticercus cellulosæ* could not be correct, for the reason that *C. cellulosæ* and its tapeworm stage, *Taenia solium*, are exceedingly rare in the United States. Probably not more than a dozen cases of pork measles are found annually at any of the large stations, where the number of hogs slaughtered amounts to hundreds of thousands. It was unbelievable that a parasite so rare in its usual host should be so common in sheep. A few days spent in studying numerous specimens obtained at the abattoirs in Chicago developed the fact that the sheep-measle parasite was certainly not *C. cellulosæ*, though in certain characters they were very similar. In some details of structure the muscle cysticerci

resembled *C. tenuicollis*, but in other respects the two forms did not agree. Accordingly an experiment was undertaken to determine whether the parasites would develop in dogs and whether the tapeworms, if any developed, would prove to be *T. hydatigena* (the tapeworm corresponding to *C. tenuicollis*; also known as *T. marginata*, the marginate tapeworm of the dog), as affirmed by Chatin (1886a), who stated that he had obtained *T. marginata* by feeding mutton cysticerci to dogs, or whether they would prove to be some other species. Seven dogs were under observation in 1912. Five of these were fed cysticerci from sheep muscle, while two, as controls, were fed *C. tenuicollis* from the omentum or mesenteries of sheep. With three exceptions, as noted below in the records of the experiment, the dogs were given a dose of castor oil and the feces examined for the presence of parasite eggs before the cysticerci were fed. During the experiment the dogs were nourished on dog biscuits, corn-meal mush, and some cooked meat but no mutton and were confined in separate kennels.

Dog No. 1.—A grayish brown young female. Fed muscle cysticerci from sheep. Feces were not examined before feeding cysts.

- March 25. Fed 1 cyst from heart muscle of sheep.
- March 27. Fed 1 cyst from heart muscle of sheep—probably dead.
- March 28. Fed 3 cysts from heart muscle of sheep.
- March 29. Fed 3 cysts from heart muscle of sheep.
- March 30. Fed 3 cysts from heart muscle of sheep—1 probably dead.
- April 1. Fed 1 cyst from diaphragm of sheep—probably dead.
- April 2. Fed 1 cyst from body muscle of sheep.
- April 3. Fed 2 cysts from heart muscle of sheep.
- April 24. Fed 1 cyst from heart muscle of sheep.
- April 29. Fed 2 cysts from heart muscle of sheep.
- May 2. Fed 1 cyst from heart muscle of sheep.
- May 21. Fed 1 cyst from heart muscle of sheep.
- May 22. Fed 1 cyst from heart muscle of sheep.
- May 24. Fed 2 cysts from heart muscle of sheep.

Total . . 23 cysts.

June 22. Eggs of *Toxascaris* and a tapeworm segment found.

June 27. Tapeworm segments found in feces.

July 24. Chloroformed. About 25 individuals of *Toxascaris* in upper half of jejunum. Seven tapeworms, all with gravid segments, in ileum. Heads attached near upper end of ileum, about 65 cm. from ileocecal valve. Length of tapeworms, 45 to 55 cm.

Dog No. 2.—A white-and-tan young female. Fed *Cysticercus tenuicollis* from peritoneum of sheep. Feces were not examined before feeding cysts.

- |                        |                      |
|------------------------|----------------------|
| April 5. Fed 3 cysts.  | May 10. Fed 1 cyst.  |
| April 9. Fed 4 cysts.  | May 28. Fed 5 cysts. |
| April 11. Fed 1 cyst.  |                      |
| April 18. Fed 7 cysts. | Total . . 21 cysts.  |

June 22. Eggs of tapeworm and *Toxascaris* eggs found.

July 11. Two tapeworm segments found in feces.

July 26. Chloroformed. Numerous individuals of *Toxascaris* in jejunum and duodenum. Nine tapeworms with gravid segments; one of the tapeworms was about 110 cm. long. The tapeworms were attached about 8 cm. below the pylorus, 80 cm.

from the ileocecal valve, and the posterior ends of the worms extended to within 40 cm. of the ileocecal valve.

Dog No. 3.—A young black-and-white female. Fed muscle cysticerci from sheep. March 29. Received one-half ounce of castor oil at 5 p. m. March 30. Feces were examined with negative results.

April 5. Fed 1 cyst from myocardium.  
April 6. Fed 3 cysts from myocardium.  
April 10. Fed 3 cysts from myocardium.  
April 11. Fed 6 cysts from myocardium.  
April 13. Fed 1 cyst from myocardium.  
April 29. Fed 1 cyst from myocardium.  
May 2. Fed 1 cyst from myocardium.  
May 21. Fed 1 cyst from myocardium.  
May 23. Fed 1 cyst from myocardium.  
May 24. Fed 3 cysts from myocardium.

Total... 21 cysts.

June 11. Feces examined but no eggs found.

No segments or eggs have been found (prior to July 22).

July 22. Chloroformed. Four tapeworms attached 25 to 35 cm. from the ileocecal valve, one of them with gravid segments, about 45 cm. long when extended, other three not over 2 to 5 cm. long. Three very short tapeworms in cecum. In large intestine a string of about 10 gravid segments. Total number of tapeworms, seven. Three individuals of *Toxascaris* in jejunum.

Dog No. 4.—A young red male. Fed muscle cysticerci from sheep. March 29. Received one-half ounce of castor oil at 5 p. m. March 30. Feces were examined and *Toxascaris* eggs found.

April 18. Fed 1 cyst from myocardium of sheep.  
April 19. Fed 1 cyst from cheek muscle of sheep.  
April 23. Fed 16 cysts—3 from myocardium and 13 from muscles of sheep.  
Hooks were well developed.  
April 24. Fed 1 cyst from myocardium of sheep.  
May 2. Fed 1 cyst from myocardium of sheep.  
May 15. Fed 1 cyst from myocardium of sheep.  
May 21. Fed 1 cyst from myocardium of sheep.  
May 24. Fed 2 cysts from muscles of sheep.

Total... 24 cysts.

June 11. Eggs of *Toxascaris*, but no tapeworm eggs found.

June 27. Three broken tapeworm segments found in feces.

July 24. Chloroformed. Two individuals of *Toxascaris* in upper part of jejunum. Sixteen or seventeen tapeworms extending down into lower part of colon. None attached more than 4 cm. above ileocecal valve. One attached in cecum. None with gravid segments. Length, 20 to 50 cm.

Dog No. 5.—A medium-sized brindled male. Fed muscle cysticerci from sheep. March 29. Received one-half ounce of castor oil at 5 p. m. March 30. Feces were examined with negative results.

April 23. Fed 20 cysts from muscles of sheep. Hooks were well developed.  
April 24. Fed 1 cyst from myocardium.  
May 2. Fed 1 cyst from myocardium.  
May 15. Fed 1 cyst from myocardium.  
May 21. Fed 1 cyst from myocardium.  
May 24. Fed 2 cysts from muscles of sheep.

Total.. 26 cysts.

June 11. Tapeworm eggs and eggs of *Toxascaris* were found in feces.

June 19. Two or three segments found in feces.

July 24. Chloroformed. No tapeworms found. Numerous dead fly larvæ in colon and small intestine. Numerous *Toxascaris* in upper part of jejunum and in duodenum.

Dog No. 6.—A medium-sized white male. Fed muscle cysticerci from sheep. March 29. Received one-half ounce of castor oil at 5 p. m. March 30. Feces were examined and *Toxascaris* eggs found.

April 23. Fed 20 cysts from muscles of sheep. Hooks well developed.

April 24. Fed 1 cyst from myocardium.

May 2. Fed 1 cyst from myocardium.

May 15. Fed 1 cyst from myocardium.

May 21. Fed 1 cyst from myocardium.

May 24. Fed 2 cysts from muscles of sheep.

—  
Total.. 26 cysts.

June 11. Tapeworm eggs and eggs of *Toxascaris* were found in feces.

June 19. Two tapeworm segments were found in feces.

July 26. Chloroformed. Eight or nine tapeworms with gravid segments, one of them measuring 1 meter in length. Heads attached 135 cm. above the ileocecal valve, and posterior ends of the worms extending to a distance of 55 cm. from the ileocecal valve. Numerous individuals of *Toxascaris* in jejunum and in duodenum.

Dog No. 7.—A medium-sized black-and-white spotted female. Fed *Cysticercus tenuicollis* from peritoneum of sheep. Feces were not examined before feeding cysts.

April 9. Fed 4 *Cysticercus tenuicollis*

April 18. Fed 7 *Cysticercus tenuicollis*.

May 28. Fed 7 *Cysticercus tenuicollis*.

—  
Total.. 18.

June 22. Feces show a few young tapeworm segments.

July 11. Found several portions of tapeworms; each portion contained from 2 to 20 segments.

July 26. Chloroformed. Three or four individuals of *Toxascaris* in duodenum and jejunum. Ten tapeworms with short strobila not over 10 mm. long in duodenum.

In continuation of the experiment with the dogs another experiment was undertaken for the purpose of recovering the cystic stages of the tapeworms. Ten lambs were purchased from a lot of thirty-nine, the remainder of which were slaughtered at one of the packing houses in Chicago and found to be free on post-mortem examination from both muscle cysticerci and *Cysticercus tenuicollis*. One of the ten died shortly after purchase and consequently was not used in the experiment. The sheep were kept during the experiment in floored and covered pens in one of the sheep barns at the Union Stock Yards, Chicago, and were fed dry hay and occasionally oats and received water piped from the water mains. The identity of the various lambs was maintained by numbered ear tags.

Lamb No. 1.—July 24. One half of a gravid segment from a tapeworm out of dog No. 1 (a dog which had been fed muscle cysts) was cut in pieces and given in a drench with water.

August 7. Dr. Day reported that lambs Nos. 1, 2, 3, and 5 were more or less sick but would probably recover.

August 21. Is very thin and has a diarrhea, but is feeding well.

October 15 (eighty-three days after feeding). Chloroformed. In poor flesh, very little fat. Cysticerci were found in the panniculus carnosus. Forty-two degenerate cysts were counted in the diaphragm; ten degenerate cysts in the wall of the esophagus. Several cysts in anterior lobes of lungs, 2 to 3 mm. in diameter; contents caseous. One contained a small dead cysticercus, 1 mm. in diameter; rudiment of head present. Numerous small degenerate cysts in heart. Numerous cysticerci in muscles of mastication; some living, others degenerate. A few nodules in the wall of the rumen, and one in the wall of the fourth stomach, 2 to 4 mm. in diameter, hard, shotlike, with thick wall and cheesy contents. No cysticerci were found in these cysts. Nodules present on wall of cecum, probably *Oesophagostomum*. No cysticerci found in these nodules. Many degenerate cysts among those present in the musculature in various parts of the body. The sizes of 13 live cysts measured in situ were as follows, in millimeters: 9 by 3.5, 8 by 3, 7 by 4, 7 by 3, 6 by 3, 5 by 4, 4 by 2.5, 5 by 3, 7 by 4, 8 by 4, 8 by 3, 6 by 2.5, and 9 by 4. A cyst 5 or 6 mm. in diameter with thick leathery capsule contained a live cysticercus which was active under the microscope. This cysticercus was not fully developed, only the blade of the hooks being formed. Other cysticerci showed fully developed hooks, and cysticerci from degenerate cysts showed in some cases hooks not yet fully formed.

Lamb No. 2.—July 26. A gravid segment from a tapeworm out of dog No. 6 (a dog which had been fed muscle cysts) was given in a drench with water.

August 7. Dr. Day reported that lambs Nos. 1, 2, 3, and 5 were more or less sick but would probably recover.

August 17 (twenty-two days after feeding). This animal died, but its death was not reported until two days later, when decomposition was so far advanced that Dr. Day did not attempt a post-mortem examination.<sup>1</sup>

Lamb No. 3.—July 26. A gravid segment from a tapeworm out of dog No. 6 (a dog which had been fed muscle cysts) was given in a drench with water.

August 7. Dr. Day reported that lambs Nos. 1, 2, 3, and 5 were more or less sick but would probably recover.

August 18 (twenty-three days after feeding). This animal died. Decomposition was far advanced the following day when a post-mortem examination was made, but some of the masseter muscle and some of the muscle of a hind leg were obtained. Dr. Day reports that cysts in the masseter muscle were quite well formed and contained a tiny white spot just visible to the eye. Microscopic examination by Dr. Day showed that the head was not well formed, but papillæ were evident on the caudal bladder.

Lamb No. 4.—July 24. A gravid segment (cut in pieces) from dog No. 1 (a dog which had been fed muscle cysticerci) was given in a drench with water.

August 7. In very bad condition; probably will die.

August 11 (eighteen days after feeding). Dead.

August 12. An incomplete post-mortem examination was made by Dr. Day. Advanced decomposition. A number of cysts were obtained from the masseter muscles.

Lamb No. 5.—July 24 a gravid segment from a tapeworm out of dog No. 3 (a dog which had been fed muscle cysticerci) and on July 26 two gravid segments from a tapeworm out of dog No. 6 (a dog which had been fed muscle cysticerci) were given in a drench with water, a total of three segments.

August 7. Dr. Day reported that lambs Nos. 1, 2, 3, and 5 were more or less sick but would probably recover.

<sup>1</sup> In prior publications (Ransom, 1913, p. 78; 1913, p. 31) it was erroneously stated that all of the lambs which had been fed eggs of the muscle cyst tapeworm showed tapeworm cysts in the muscles. The condition in lamb No. 2, of course, was not determined, as no autopsy was made on this animal. The statement (Ransom, 1913, p. 31) that the lambs died in 14 to 22 days after feeding is also inaccurate. It should be 13 to 23 days.



August 12 (ten days after feeding). Dead. Post-mortem examination by Dr. Day the following morning showed a large number of cystic parasites in the masseter muscles, heart, tongue, and diaphragm. There were also numerous cystic parasites in the skeletal muscles and a few hemorrhagic spots.

Lamb No. 6.—July 24, four segments from tapeworms out of dogs Nos. 1 and 3 (dogs which had been fed muscle cysticerci), two segments from each dog, and July 26 six segments from tapeworms out of dog No. 6 (a dog which had been fed muscle cysticerci) were given in a drench with water, a total of ten segments.

August 5. Appears ill and out of condition.

August 6 (thirteen days after feeding). Dead. Post-mortem by Dr. Day showed that the parasites had already migrated to the muscles, and were found as very minute cysts, more numerous in the heart and masseter muscles than elsewhere. There were about 25 c. c. of fluid in the pericardium. The heart was very thickly studded with minute cysts. There were about 350 c. c. of fluid in the peritoneal cavity. A careful examination of the fluid was made, but no parasites were found. The liver appeared normal.

Lamb No. 7.—July 26. A gravid segment from a tapeworm out of dog No. 2 (a dog which had been fed *Cysticercus tenuicollis* from the peritoneum of sheep) was given in a drench with water.

August 21. Reported by Dr. Day as doing well.

October 18 (eighty-four days after feeding). Chloroformed. Animal in poor flesh. Twelve to fifteen cysts on omentum and mesenteries, two of which are alive, the others degenerate. One degenerate cyst under peritoneum in pelvic cavity. Degenerate cysts vary in size up to a maximum of 20 mm. in diameter. Contain dead cysticerci, a small amount of colorless serous fluid and flocculent debris or a greenish, caseous material. The live cysticerci measure 8 by 15 mm., and show the usual macroscopic characters of *Cysticercus tenuicollis*. A few degenerate cysticerci of small size on the surface of the liver. No cysticerci in the muscles, lungs, or other organs, except as noted above. *Oesophagostomum* nodules on the intestine.

Lamb No. 8.—July 26. Ten gravid segments from a tapeworm out of dog No. 2 (a dog which had been fed *Cysticercus tenuicollis* from the peritoneum of sheep) given in a drench with water.

August 21. Reported by Dr. Day as doing well.

October 17 (eighty-three days after feeding). Chloroformed. Animal in poor flesh. A considerable number of small degenerate cysticerci on surface and in depths of liver. About 25 degenerate cysts on omentum and mesenteries. One live cysticercus on omentum about 12 mm. in diameter shows the usual macroscopic characters of *Cysticercus tenuicollis*. One degenerate cyst on tendinous portion of diaphragm (abdominal surface). Small nodules in lungs, one of which contained a young dead cysticercus showing under the microscope transverse ridges on the cuticle of the caudal bladder. Synthetocaulus nodules also present on the lungs. Several pockets in the lungs with fibrous walls containing greenish pus. The contents of these pockets were examined, but no cysticerci were found. Heart and muscles were free from parasites. A cyst from the omentum, 8 mm. in diameter, with thick fibrous wall contains a dead cysticercus with evaginated head and bladder about 3 mm. in diameter. Two cysts from the omentum or mesentery, 5 and 6 mm. in diameter, respectively, contain each a dead cysticercus and a small amount of colorless serous fluid and flocculent debris. The other degenerate cysts are similar, except the contents in some are greenish, caseous. Their size varies from 2.5 to 10 mm., and all have thickened walls  $\frac{1}{2}$  to  $\frac{3}{4}$  mm. thick. The degenerate cyst from the tendinous portion of the diaphragm is flattened, 8 mm. in diameter. Its wall is thin, and it contains a dead *Cysticercus tenuicollis* and a small amount of serous fluid and white flocculent matter.

Lamb No. 9.—A check animal, not fed with tapeworm segments.

October 18. Chloroformed. In poor flesh. Free from parasites except *Oesophagostomum* nodules on the intestines.

The following experiments relating to the possibility of the development of sheep-measle tapeworms in man have been carried out, the writer being the subject.

On March 6, 1913, a cysticercus about 5 mm. in diameter, and March 14 another cysticercus of similar size, both from sheep hearts, were swallowed. Both cysticerci were alive and in good condition, exhibiting lively contractions of the caudal bladder when viewed under the microscope. On March 28 eight fully developed cysticerci were isolated from a sheep carcass heavily infested with *Cysticercus ovis* and swallowed. These cysticerci were apparently in good condition and were undoubtedly alive, as they showed active movements under the microscope. No signs of tapeworm infestation have appeared in the case of the writer.

#### SUMMARY OF LIFE-HISTORY EXPERIMENTS

Five dogs were each fed from 21 to 26 muscle cysticerci from sheep on various dates between March 25 and May 24. Subsequent to June 11, tapeworm eggs or segments were demonstrated in the feces, or tapeworms were found post-mortem in the case of all five dogs. No tapeworms were found in one of the dogs (No. 5) post-mortem, but a month earlier this dog had shown tapeworm eggs and segments in the feces. In the case of two of the dogs (Nos. 5 and 6) it was evident that the tapeworms had reached egg-producing maturity within seven weeks, as the earliest feeding of cysticerci was on April 23, eggs being demonstrated in the feces on June 11. The number of tapeworms recovered varied from 7 to 16.

Two dogs were fed *Cysticercus tenuicollis*, 18 and 21 cysticerci, respectively, between April 5 and May 28. The first tapeworm eggs were found in the feces on June 22. On post-mortem examination 9 tapeworms were found in one dog and 10 in the other.

Six lambs (Nos. 1 to 6) were fed with gravid segments of tapeworms from the dogs which had been fed *Cysticercus ovis*, two (Nos. 7 and 8) with gravid segments of tapeworms from one of the dogs which had been fed *C. tenuicollis*, and one (No. 9) was retained under the same conditions as the others but without receiving any cysticerci. Lambs Nos. 1 to 6 received  $\frac{1}{2}$  to 10 segments, and lambs Nos. 7 and 8, 1 and 10 segments, respectively. Of the former all but the one receiving half a segment died in 13 to 23 days after feeding, the one receiving 10 segments being the first to die, followed by one receiving 1 segment (death in 18 days), then by one receiving 3 segments (death in 19 days), then by two more receiving 1 segment each (death in 22 and 23 days, respectively), leaving the lamb which received half a segment to survive until killed—83 days after feeding. Both of the lambs fed with segments of *Taenia hydatigena* (adults

of *C. tenuicollis*) survived until killed at the close of the experiment. All but one of the lambs (No. 2), which died 22 days after feeding, were examined post-mortem.

Omitting this lamb from consideration, all of the lambs which received segments from the tapeworms produced by feeding muscle cysticerci showed cysticerci in their muscles when examined. Those found in the lamb which died 13 days after feeding were very small; those in the lamb which died 23 days after feeding were somewhat farther along in development, the beginnings of the head being already evident. Eighty-three days after feeding, the muscle cysticerci were found to have reached full development; some which had fully developed were already more or less degenerated, and some were found which had begun to degenerate before they reached their full development. In addition there were present live cysticerci which had not yet fully developed.

The lambs which had been fed segments of *Taenia hydatigena* showed a few *Cysticercus tenuicollis*, most of which were degenerate. In both animals there were small degenerate cysticerci on the liver. There were no visible lesions of the liver in the lambs fed segments of *T. ovis*. No *C. tenuicollis* was found in any of the lambs fed segments of *T. ovis*, and no *C. ovis* in the lambs fed segments of *T. hydatigena*. The check lamb showed neither *C. tenuicollis* nor *C. ovis*, and neither of these parasites was found at the post-mortem inspection of the remainder of the lot from which the experiment sheep had been selected.

Since these experiments show that muscle cysticerci in sheep resembling *Cysticercus cellulosae* and corresponding to the form described by Cobbold as *C. ovis* develop into tapeworms when swallowed by dogs, it has been definitely proved that these cysticerci are not *C. cellulosae*. The adult of *C. cellulosae* (*Taenia solium*) does not occur in dogs; moreover, the tapeworms which were produced in the dogs are quite different from *T. solium*. Furthermore, the experiments prove that the muscle cysticercus and its adult stage are specifically distinct from *C. tenuicollis* and *T. hydatigena*. It appears that the ingestion of one or more gravid segments of *T. ovis* is likely to prove fatal to sheep.

Attempts to produce tapeworms in man by feeding mutton cysticerci failed. On three occasions live mutton cysticerci were swallowed by the writer, a total of 10 cysticerci being ingested. No evidence of tapeworm infestation has since appeared. This experiment tends to prove that *Cysticercus ovis* is not transmissible to man.

#### SYNOPSIS OF LIFE HISTORY

The adult of *Cysticercus ovis* is a tapeworm (*Taenia ovis*) which occurs in the intestine of dogs. Since the parasites which live on dogs as a rule also thrive on wolves, and, since coyotes and other wolves frequently

devour sheep, it is quite likely that *T. ovis* also occurs in coyotes and other wolves as well as in dogs. In view of the fact, however, that dogs come in much closer relations with sheep it seems quite evident that dogs are chiefly responsible for the transmission of the parasite to sheep. It is possible though rather unlikely that the tapeworm occurs in other carnivores than dogs and wolves. There is little likelihood that the parasite is transmissible to man, and for all practical purposes its nontransmissibility to man may be considered an established fact. No such tapeworm has been reported from man, and, moreover, there are no authentic cases of the occurrence in man of any dog tapeworm belonging to the genus *Taenia*. Furthermore, Chatin has noted that the swallowing of muscle cysticerci from sheep failed to result in infestation in his case. The present writer, as already noted, has likewise on three occasions swallowed live and active muscle cysticerci from sheep without resulting infestation (p. 26).

Following the ingestion of the eggs of the tapeworm by sheep, the parasites reach the muscles in less than 13 days; they either do not pass through the liver or, unlike *Cysticercus tenuicollis*, leave no trace of their passage through this organ. In less than three months (83 days) the cysticerci reach their full development. As early as seven weeks after the ingestion of the cysticercus by a dog, its development to the mature egg-producing tapeworm may be complete. The development therefore appears to be somewhat more rapid than in the case of *Taenia hydatigena*, which was found by Leuckart (1856a) to require from 10 to 12 weeks. No doubt, however, the period required for development is subject to great variation, and though seven weeks is perhaps near the minimum for *T. ovis*, the period very likely may be greatly prolonged, as has been noted by Hall (1911, p. 510) in the case of the gid tapeworm.

#### ZOOLOGICAL DESCRIPTION OF THE SHEEP-MEASLE PARASITE

***Taenia ovis*** (Cobbold, 1869) Ransom, n. comb., 1913.

1869: *Cysticercus ovis* Cobbold, 1869a, p. 30, fig. 2 (in *Ovis aries*; England).

1873: *Cysticercus ovipariens* Maddox, 1873a, p. 245-253, pl. 18, figs. 1-15, 17-18, pl. 19, fig. 1 (in *Ovis aries*; England).

1878: *Cysticercus cellulosae* of Küchenmeister, 1878, in Küchenmeister and Zürn, 1878-1881a, p. 104 (apparent misdetermination of *C. ovis*; in *Ovis aries*; Germany).

1885: *Cysticercus tenuicollis* of Chatin in Railliet, 1885a, p. 234 (apparent misdetermination of *C. ovis*; in *Ovis aries*; France).

1886: *Cysticercus oviparus* Leuckart 1886d, p. 498 (for *C. ovipariens*).

1913: *Taenia ovis* (Cobbold) Ransom, 1913.

#### SPECIFIC DIAGNOSIS OF TAENIA.

*Larval stage*.—An oval cysticercus (Pl. II, fig. 1) 3.5 by 2 mm. to 9 by 4 mm. in diameter. Head and neck invaginated from the wall of the caudal bladder not at one end but about midway between the ends. Membrane of bladder very thin; with small mammillate projections; not corrugated transversely (fig. 5 and fig. 6, a). Neck transversely corrugated, coiled spirally when invaginated, 1 to 5 mm. long when evaginated. Head 500 to 800 $\mu$  in width; suckers oval, 240 to 320 $\mu$  in diameter; rostel-

lum prominent, 275 to 375 $\mu$  in diameter. Hooks (fig. 6) 24 to 36 in number, commonly 28 to 32, arranged in a double crown of alternating large and small hooks. Hooks rather slender (more slender and more lightly built than those of *Cysticercus cellulosae*); dorsal root of large hooks longer than the blade; in both large and small hooks a more or less well-marked outward curving of the dorsal border of the hook in the transitional region between the blade and dorsal root; ventral root of small hooks transversely enlarged, not bifid but sometimes presenting a faint median groove. Large hooks 156 to 188 $\mu$  long, average 173 $\mu$ ; blade (from point of blade to tip of ventral root measuring in a straight line) 68 to 84 $\mu$ , average 78 $\mu$  (based on measurements of 24 hooks, fully developed or nearly so, from 10 cysticerci taken from various sheep and 13 hooks from the heads of 4 adult worms). Small hooks 96 to 128 $\mu$  long, average 113 $\mu$ ; blade (from point of blade to tip of ventral root measuring in a straight line) 48 to 60 $\mu$ , average 57 $\mu$  (based on measurements of 26 hooks, fully developed or nearly so, from 11 cysticerci taken from various sheep and 10 hooks from the heads of 4 adult worms).<sup>1</sup>

Calcareous corpuscles numerous in the neck, less numerous in the head, and very rare in the caudal bladder.

*Adult stage* (Pl. II, fig. 3; text figs. 7, 8, 9, and 10).—Length of living worms with gravid segments, 45 to 110 cm. Length (preserved material), 14 to 53 cm.; maximum width, 4 to 8.5 mm.; terminal segments, 2.5 to 15 mm. long by 4 to 6 mm. broad, usually longer than broad (measurements of 17 specimens with gravid segments). Strobila tends to twist in the form of a spiral. Head 0.8 to 1.25 mm. in breadth; neck, 0.65 to 0.9 mm. wide (measurements of 26 preserved specimens). Rostellum 375 to 430 $\mu$  in diameter (8 specimens). Suckers 270 to 320 $\mu$  in diameter (4 specimens). Number, arrangement, shape, and size of hooks as in larva. Segments with convex lateral borders, in consequence of which the edge of the strobila commonly presents a scalloped outline whose regularity is broken by the protuberant genital papillæ. The genital papillæ are irregularly alternate and are situated posterior of the middle of the segment; in gravid segments they may attain a diameter of over 1 mm. and a height of three-fourths of a mm. Genital sinus large, varying in depth and width up to a maximum of about 400 $\mu$ . Cirrus pouch 450 to 550 $\mu$  long; inner end near the outer side of the ventral longitudinal excretory vessel. The testicles are distributed in an area which extends anteriorly to the anterior limits of the segment and laterally to the longitudinal excretory vessels. This area is bounded posteriorly by a curved line which in sexually mature segments intersects the median line at a distance from the anterior border of the segment varying from a little more than a third to a little less than half the length of the segment and intersects the longitudinal excretory vessels a short distance in front of the posterior border of the segment, thus leaving an approximately semicircular space entirely free from testicles, most of which is occupied by the ovary. Behind the latter is the so-called yolk gland. The ovary is bilobed, the

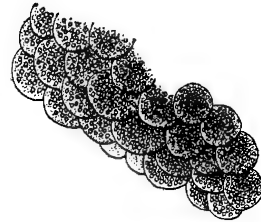


FIG. 5.—*Cysticercus ovipariens* (= *C. ovis*): Papillæ on caudal bladder,  $\times 160$ . (After Maddox, 1873a, pl. 18, fig. 15.)

<sup>1</sup> Measurements of 26 hooks.

Member.	Larva.	Adult.	Member.	Larva.	Adult.
Large hooks:			Small hooks:		
Entire.....	$\mu$ . 156 to 188	$\mu$ . 160 to 184	Entire.....	$\mu$ . 96 to 120	$\mu$ . 104 to 128
Average.....	173	173	Average.....	112	116
Blade.....	76 to 80	68 to 84	Blade.....	52 to 60	48 to 60
Average.....	79	75	Average.....	57	57

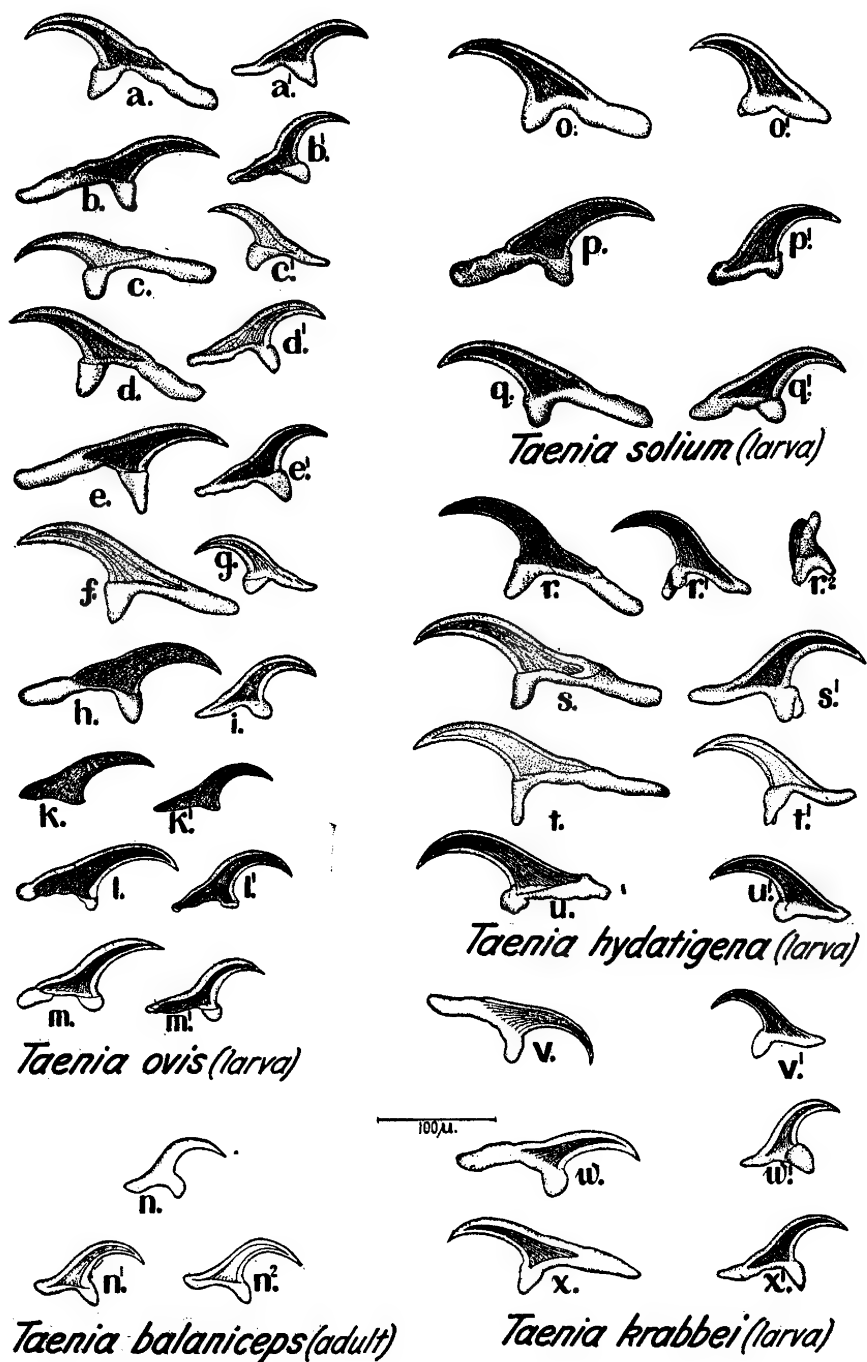


FIG. 6.—Hooks of *Taenia ovis*, *T. hydatigena*, *T. solium*, *T. balaniceps*, and *T. krabbei*. Large and small hooks designated by the same letters are from the same heads. The hooks shown in v and v' are from the type material of *T. krabbei* (B. A. I. No. 19352). Enlarged. (Original.)

antiporal lobe being slightly larger than the other. Laterally the ovary extends to the testicular field, but anteriorly is separated from it by a space which is greatest in

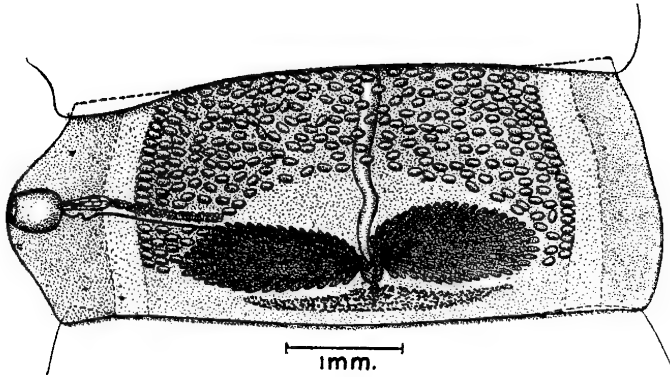


FIG. 7.—Sexually mature segments of *Taenia ovis*. Enlarged. (Original.)

the median line. Posteriorly the testicular field extends beyond the posterior limits of the ovary but slightly, if at all, and falls short of a transverse line drawn through the posterior border of the yolk gland. Gravid uterus (figs. 9 and 10) with 20 to 25 lateral branches from the median stem. Eggs (embryophores) oval, 30 by 24 to 34 by 28 $\mu$  in diameter.

HOSTS.—Larval stage: Sheep (*Ovis aries*); goat (*Capra hircus*).<sup>1</sup> Adult stage: Dog (*Canis familiaris*).

LOCATION.—Larval stage: Muscles (heart, voluntary muscles, esophagus), more rarely lungs, wall of stomach (?), and kidneys (?). Adult stage: Lumen of small intestine.

LOCALITIES.—England, France, Germany, Algeria, German Southwest Africa, New Zealand, and United States.

TYPE SPECIMENS.—Probably not in existence.

#### REMARKS ON MORPHOLOGY AND COMPARISON WITH OTHER SPECIES

The larval stage of the sheep-measle tapeworm somewhat resembles *Cysticercus cellulosae* in its general morphology. The spirally disposed neck and head and the mammillate surface of the caudal bladder suggest the pork cysticercus. The smaller average size and more delicate structure of the cysticercus and the shape and number of the hooks, however, differentiate it quite clearly from *C. cellulosae*. The hooks are somewhat slighter in build, have smaller blades, and are different in outline; the number commonly exceeds the usual number found in *C. cellulosae*, though the limits of variation in number are such in the two forms (24 to 32 in *C. cellulosae*, according to various authors, and 24 to 36 in *C.*

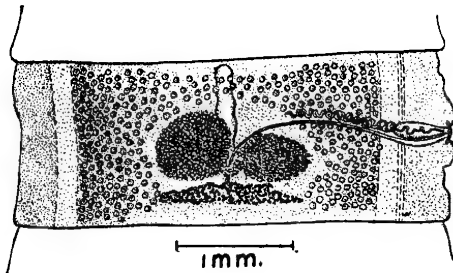


FIG. 8.—Sexually mature segments of *Taenia hydatigena*. Enlarged. (Original.)

<sup>1</sup> This record is based on a specimen in the collection of the Bureau of Animal Industry collected in April, 1912, from the heart of a goat about 2 years old, origin unknown, slaughtered at one of the abattoirs in Kansas City, Mo.

*ovis*) that a definite diagnosis can not be made in individual cases on the basis of the number of hooks if this number happens to be 32 or less.

Apart from the fact that its normal location is in muscle and not on serous membranes, *Cysticercus ovis* may be distinguished from *C. tenuicollis* by its smaller size, the different relationship of the head and neck

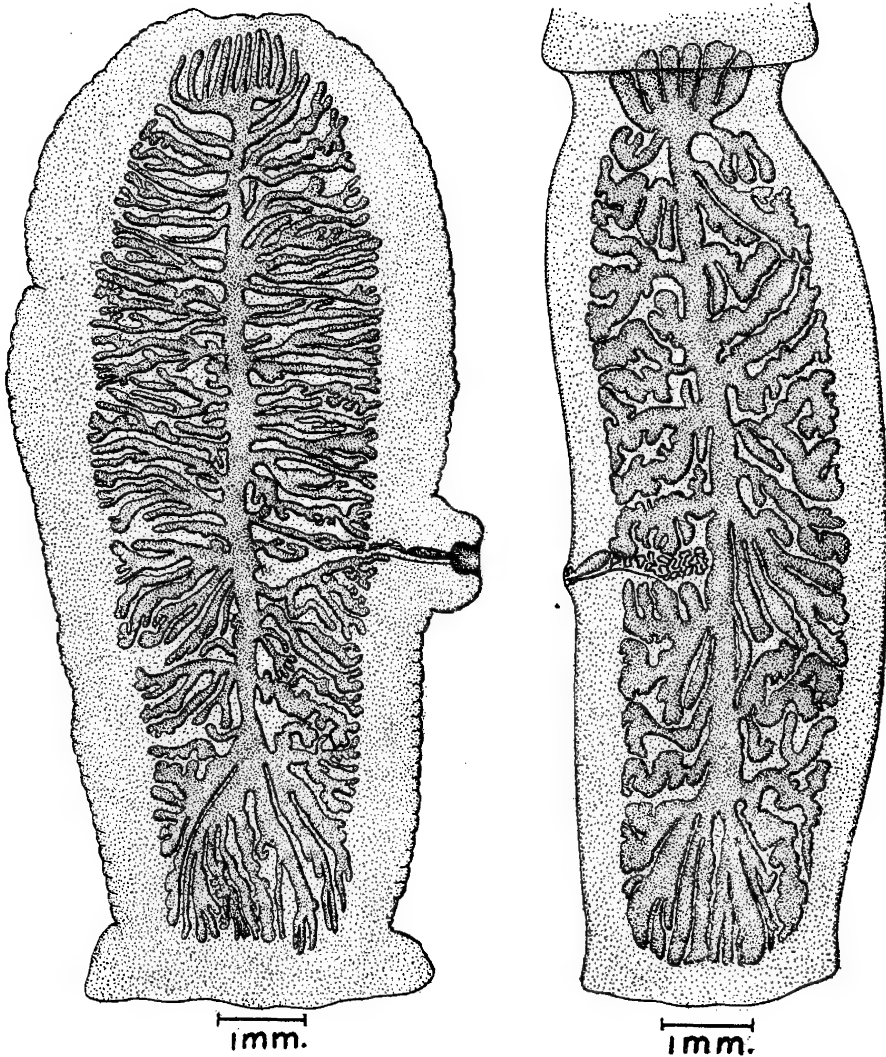


FIG. 9.—Gravid segments of *Taenia ovis*. Enlarged. (Original.)

FIG. 10.—Gravid segments of *Taenia hydatigena*. Enlarged. (Original.)

to the caudal bladder, the presence of mammillate projections on the surface of the caudal bladder instead of transverse corrugations, and the different size of the hooks. In *C. tenuicollis* the head and neck are invaginated from one end of the caudal bladder instead of from the side, as in *C. ovis* (Pl. II, figs. 1 and 5). The mammillate projections on the surface of the caudal bladder of *C. ovis* (figs. 5 and 11) are very much in



contrast to the transverse rugæ on the caudal bladder of *C. tenuicollis* (fig. 12).

As the number of hooks of *Cysticercus tenuicollis* has been found by various observers to vary from 26 to 44, an accurate distinction between this form and *C. ovis* which would be applicable in all cases can not be drawn on the basis of the number of hooks, though, as a rule, the number of hooks found in *C. ovis* is less than the number commonly present in *C. tenuicollis*. There is also an overlapping in the size of the hooks,

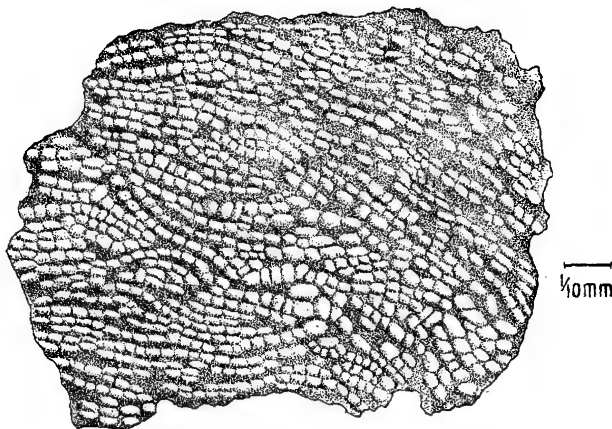


FIG. 11.—Surface of caudal bladder of *Cysticercus ovis* showing papillæ. Enlarged. (Original.)

the recorded limits for the large hooks being 170 to 220 $\mu$  in *C. tenuicollis* (larva and adult) and 156 to 188 $\mu$  in *C. ovis* (larva and adult), and for the small hooks 110 to 160 $\mu$  in *C. tenuicollis* (larva and adult) and 96 to 128 $\mu$  in *C. ovis* (larva and adult).

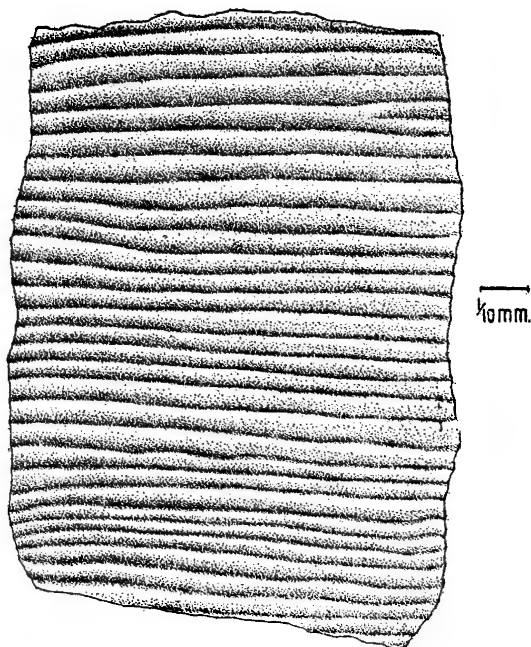


FIG. 12.—Surface of caudal bladder of *Cysticercus tenuicollis* showing transverse furrows. Enlarged. (Original.)

the average length of 37 large hooks of *T. ovis* (adult and larva) having a range of 156 to 188 $\mu$  was 173 $\mu$ , with the blade ranging from 68 to 84 $\mu$ ,

The hooks of *Cysticercus tenuicollis*, however, average considerably larger than those of *C. ovis*, both in total length and in length of blade (fig. 6). In 25 large hooks from four adult and two larval individuals of *Taenia hydatigena* (*C. tenuicollis*) ranging in length from 180 to 212 $\mu$ , averaging 197 $\mu$ , the blade varied from 72 to 108 $\mu$  in length and averaged 93 $\mu$ ; and 20 small hooks from the same specimens ranging in length from 116 to 136 $\mu$ , average 129 $\mu$ , had blades ranging in length from 60 to 76 $\mu$ , average 68 $\mu$ . The

average  $78\mu$ . The average length of 36 small hooks of *T. ovis* (adult and larva) having a range of  $96$  to  $128\mu$  was  $113\mu$ , with the blade ranging from  $48$  to  $60\mu$ , average  $57\mu$ . In form the hooks of *T. hydatigena* and *T. ovis* are very similar. The small hooks may be distinguished from each other by the fact that the ventral root, though transversely enlarged in both species, is rather deeply bifid in *T. hydatigena* (fig. 6,  $r^2$ ), a condition which is absent in *T. ovis* or at most only faintly indicated.

Of the more common tapeworms of the dog the one with which *Taenia ovis* seems most likely to be confused is *T. hydatigena* (*T. marginata*), the adult of *Cysticercus tenuicollis*. Apart from the differences exhibited by the hooks as noted above, the segments of the strobila show certain characters by which the two species may be differentiated. (Pl. II, figs. 3, 4, 5; and text fig. 6.) The strobila of *T. hydatigena* is thicker (dorso-ventrally) relatively to its other dimensions than that of *T. ovis* and the latter has a tendency to twist spirally. The segments of *T. hydatigena* have a rather regular quadrilateral form, and the edge of the strobila is comparatively straight, whereas in *T. ovis* the segments have convex lateral borders, the convexity usually being well marked, and the edge of the strobila presents a scalloped outline. The posterior margin of the segment projects more prominently in the former than in the latter species. In *T. ovis* the genital pore is in a large prominent genital papilla, and there is a large and deep genital sinus; in *T. hydatigena* the genital papilla is small and the genital sinus shallow and inconspicuous. The testicles in *T. ovis* do not extend posterior of a line drawn through the anterior border of the yolk gland parallel with the posterior border of the segment; in *T. hydatigena* they extend beyond the posterior limits of the ovary and yolk gland practically to the posterior border of the segment (figs. 7 and 8). With respect to the branching of the uterus, *T. ovis* and *T. hydatigena* are quite different, the uterus of the former having 20 to 25 lateral branches from each side of the median stem, whereas the uterus of the latter has but 5 to 8 such branches (figs. 9 and 10).

Other well-known tapeworms of the dog, such as *Taenia pisiformis* (*T. serrata*), *Multiceps multiceps* (*T. coenurus*), *Multiceps serialis* (*T. serialis*), *Echinococcus granulosus* (*T. echinococcus*), and *Dipylidium caninum*, are less likely than *T. hydatigena* to be confused with *T. ovis*. In addition to distinct morphological differences, the small size of *E. granulosus* and *D. caninum* precludes any chance of mistaking them for *T. ovis*. *T. pisiformis* may be distinguished by the large size of its hooks (the large hooks being  $225\mu$  or more in length) and the small number of lateral branches of the uterus (8 to 10). *M. serialis* may be distinguished from *T. ovis* by the fact that the hooks are considerably smaller, the recorded limits of length of the large hooks being  $135\mu$  and  $157\mu$ , that the ventral roots of the small hooks are distinctly bifid, and that the genital papillae are small and inconspicuous. *M. multiceps* has large hooks about the same in length as those of *T. ovis* but with blades longer

than half the total length of the hook; and as the genital sinus and genital papilla are very small, the two species may be readily distinguished from each other.

Of the less common or less known tapeworms of the dog the species of *Dibothriocephalus* and *Mesocestoides* are immediately to be distinguished from *Taenia ovis* by the absence of cephalic hooks and rostellum and by the location of the genital pores in the ventral median line of the segment. Likewise, the absence of hooks and rostellum distinguishes *Ophidiotaenia punica* (*Proteocephalus punicus*)<sup>1</sup> from *T. ovis*.

The remaining species of tapeworms known to occur in the dog are *Taenia balaniceps*, *T. brauni*, *T. brachysoma*, and *T. krabbei*, all of which, with the exception of the last, may be readily distinguished from *T. ovis* upon the basis of their published descriptions.

*Taenia balaniceps* Hall (1910, pp. 139-151, figs. 1-8) differs from *T. ovis* in various particulars, among which may be mentioned the following: The worm is smaller, the length of the longest specimen being only 24 cm.; the head is smaller, not exceeding  $752\mu$  in breadth, and the segments in corresponding stages of development are smaller. The hooks are smaller, 93 to  $98\mu$  being given as the limits of length of the small hooks and  $145\mu$  as the length of the large hooks (fig. 2.) The testicles extend practically to the posterior border of the segment, as in *T. hydatigena*. The lateral branches of the uterus, instead of being slender and more or less separated by intervening spaces as in *T. ovis*, are comparatively thick and are pressed close together.

*Taenia brauni* Setti, 1897 (Setti, 1897b, pp. 210-214, pl. 8, figs. 9-14), differs from *T. ovis* in that it is much smaller, its total length being from 15 to 18 cm., and the size of the posterior segments 5 or 6 mm. long by 3.5 mm. wide. *T. brauni* was described as lacking a true rostellum but as possessing a double crown of 30 hooks, the large hooks measuring 130 to  $140\mu$ , though in some cases only 95 to  $100\mu$  in length, and the small hooks usually 85 to  $90\mu$ , occasionally 70 to  $75\mu$ , in length. *T. ovis*, however, has a well-developed rostellum and hooks considerably larger than the dimensions given for *T. brauni* and is thus clearly a different species from the latter, though the two forms agree in possessing prominent genital papillæ and perhaps are similar in regard to the branches of the uterus, as Setti states that the lateral branches are numerous, slender, and perpendicular to the medium stem.

*Taenia brachysoma* Setti, 1899 (Setti, 1899c, pp. 11-20, pl. 1, figs. 1-9), is also a much smaller species than *T. ovis*, specimens with gravid segments being not over 10 cm. long and not over 3 mm. in maximum width. The number of hooks is 30 to 32. The large hooks measure 135 to  $145\mu$  and the small hooks 95 to  $105\mu$  in length, the former thus being considerably smaller than in *T. ovis*, and the latter averaging smaller. The ventral roots of the small hooks are described as having a median groove, thus presenting a condition intermediate between simple and

<sup>1</sup> This species, as pointed out by Hall (1910, p. 146), is probably not a true parasite of the dog.

bifid, at the same time twisted so that the lateral axis tends to lie in the plane of the blade and dorsal root.<sup>1</sup>

The genital papillæ are small and inconspicuous in *T. brachysoma* and the genital sinus measures not over  $170\mu$  in maximum depth. The lateral branches of the uterus number only 10 to 12 on each side of the median stem.

*Taenia krabbei* Moniez (1879c, pp. 161-163; 1880a, pp. 44-50, 56, pl. 1, figs. 12-14, pl. 2, figs. 4-7) produced in a dog by feeding cysticerci from the muscles of reindeer is described as much longer, wider, and thicker than *T. coenurus* and *T. serrata* and has much wider segments proportional to their length, but its head is more delicate. It is also much larger than *T. marginata*, the head is larger, and the segments are wider in proportion to their length. The genital pores are located in large papillæ, often attaining a diameter of 1 millimeter, equal to the length of the contracted segment. The cysticercus according to Moniez is much smaller than the cysticercus of *T. solium*. The number of hooks varies from 26 to 34. The caudal vesicle, compared to the size of the head and neck, is very slightly developed and does not contain much fluid. The orifice of invagination of the cysticercus may be either at one pole or at one side. The invaginated head and neck commonly curve spirally as in *Cysticercus cellulosæ*, but to a less degree. The size of the hooks is not given by Moniez.

If the stated magnification of a drawing by Moniez is correct, the length of the large and small hooks would be about  $215\mu$  and  $160\mu$ , respectively, but inasmuch as the large hooks of *C. tenuicollis*, shown in another drawing, measure, according to the magnification given, about  $350\mu$  in length, whereas the maximum recorded length is less than  $250\mu$ , it is not unlikely that there has been some error also in stating the magnification of the drawing of the hooks of *T. krabbei*, so that sizes calculated from the magnifications of Moniez's drawings can not be considered at all accurate. Cysticerci in the Bureau of Animal Industry Helminthological Collection found in reindeer in Alaska by Dr. D. S. Neuman and corresponding to *T. krabbei*, so far as may be determined from Moniez's description and figures, except as to the size of the hooks, have hooks (fig. 6) of the following dimensions: Large hooks 150 to  $170\mu$  in length, average  $162\mu$ , with blades 75 to  $80\mu$  long, average  $77\mu$ ; small hooks 85 to  $120\mu$  in length, average  $107\mu$ , with blades 52 to  $60\mu$  long, average  $57\mu$  (measurements based on 34 large and 34 small hooks from 8 cysticerci). The average size of the hooks is thus less than the average of the hooks in *C. ovis*, but they show no remarkable difference in form from those of the latter. Corresponding closely to Moniez's findings, the number counted on eight heads varied from 26 to 32. The invaginated head and neck of the cysticercus form a much larger structure than in *C. ovis* both actually and relatively to the size of the caudal bladder. On account of

<sup>1</sup> Setti does not make it clear whether this twisted condition is invariably present. The small hooks of *Taenia hydatigena* commonly present a similar appearance after subjection to the pressure of a cover glass.

their shriveled condition the size of the cysticerci could not be accurately determined; apparently, however, they are somewhat smaller than *C. cellulosa*, rather slender and considerably elongated. The cysticercus of *T. krabbei* is readily distinguished from *C. ovis* by its elongated form, by the fact that the orifice of invagination of the head and neck is commonly at one end of the cysticercus instead of at the side, and by the larger size of the body formed by the invaginated head and neck both actual and relatively to the size of the caudal bladder. On account of certain evident similarities, such as the prominent genital papillæ, and on account of the lack of an accurate detailed description of *T. krabbei*, no clear distinctions can be drawn between *T. krabbei* and *T. ovis*, though, no doubt, distinct differences could be found upon comparing specimens of the two species.

Since the foregoing paragraph was written some of Moniez's cotypes have been received from Prof. R. Blanchard, one specimen of the adult (B. A. I. No. 17351) and two specimens of the cysticercus (B. A. I. No. 17352). The cysticerci, considerably shrunken, measure about 2 by 3 mm. The surface of the caudal bladder is mammillated (as is also the case in the Alaskan cysticercus), and the cysticercus in this character thus resembles *Cysticercus ovis*. The number of hooks was not determined, as most of them in the one specimen dissected were lost in mounting. Two of the large hooks measured  $148\mu$  in length and had blades  $70\mu$  long. A small hook measured  $105\mu$  in length and had a blade  $60\mu$  long (fig. 6, *v*, *v'*). It has thus been determined that the sizes heretofore assigned to the hooks of *Taenia krabbei*, based on Moniez's drawings, are erroneous and the apparent discrepancy between *T. krabbei* and the Alaskan cysticercus, noted in the preceding paragraph, has been removed. The ventral root of the small hooks is transversely enlarged, but is not distinctly bifid. A tendency toward the bifid condition, however, has been observed in some instances in the Alaskan specimens. The data thus far available do not indicate a specific difference between Moniez's species and the Alaskan form, and the weight of evidence is still in favor of the correctness of the presumption that the Alaskan cysticercus and *T. krabbei* are identical. The adult specimen (B. A. I. No. 17351) corresponds closely to the drawing given by Moniez (1880a). The segments are remarkable for their great breadth, as compared with their length, and the large genital papillæ, about a millimeter in diameter, are quite conspicuous. As the strobila may be abnormally contracted in length, too much weight should not, perhaps, be placed upon the extreme shortness of the segments relative to their width as a feature by which *T. krabbei* may be distinguished from *T. ovis*. It seems probable, however, that there is a more or less marked difference in this respect between the two forms. The two posterior segments in the specimen of *T. krabbei*, which are gravid, are nearly as long as broad, measuring about 4 mm. in length by 4.5 mm. in breadth. They are considerably smaller than the gravid segments of *T. ovis*. A distinct difference

between *T. ovis* and *T. krabbei* is apparent in the gravid uterus. Instead of the 20 to 30 lateral branches seen in *T. ovis* there are in *T. krabbei* only about 10 lateral branches from each side of the median stem. It is quite clear from the brief study which has been made of the type material of *T. krabbei* that it is specifically distinct from *T. ovis*, although the similarity between the two species is very close in many respects.

#### MACROSCOPIC APPEARANCE OF CYSTICERCUS OVIS

The cyst of the fully developed undegenerated cysticercus as seen embedded in the muscles of its host is oval and varies in size from 4 by 2.5 mm. to 9 by 4 mm. or slightly larger (Pl. III, A and B). It is whitish in color and varies in transparency according to the thickness of its fibrous capsule, which may be very thin and rather transparent or comparatively



FIG. 13.—*Cysticercus ovis* (= *C. Ovis*): a, Hook,  $\times 160$ ; b, cyst containing cysticercus cut across,  $\times 2$ . (After Maddox, 1873a, pl. 18 fig. 1.)

thick and rather opaque. In transparent cysts the head and neck of the cysticercus are apparent as a small, bright, white spot showing through the wall of the cyst. Removed from its cyst the cysticercus (Pl. II, fig. 1) appears as a small oval vesicle very transparent and delicate, filled with a clear fluid, and varying in size when fully developed from 3.5 by 2 mm. to 9 by 4 mm. On one side may be seen the opaque white head and neck invaginated into the vesicle or quite commonly partially evaginated and then projecting above the surface of the vesicle. *Cysticercus ovis* is more delicate in appearance and averages

in size smaller than *C. cellulosae*. It is considerably smaller than a fully developed *C. tenuicollis*.

Degenerate cysts (fig. 13, b, and Pls. III, fig. E, and IV, fig. 2) vary in size, shape, thickness of capsule, and consistency and color of contents. The sizes of 50 degenerate cysts taken at random varied from 3.5 to 15 mm. in diameter; 7 by 4 mm. was a common size. Different authors have observed cysts varying in size from that of a millet seed to that of a bean. The shape is commonly oval or spheroidal, but may exhibit various irregularities.

The fibrous capsule of the degenerate cyst may be quite thin or relatively very thick. For example, the capsule of a cyst from the masseter muscle, measuring 7 by 4 mm., was about one-third of a millimeter thick; another cyst, 5 by 2.5 mm. in diameter, from the same muscle had a capsule about three-fourths of a millimeter thick; a cyst 10 by 7 mm. from the heart had a capsule 3 mm. thick; and the capsule of another cyst, 8 by 6 mm. in diameter, also from the heart, measured one-third of a millimeter in thickness. The cavity of the cyst is commonly irregular in shape and contains besides the cysticercus a mass of caseous, caseo-calcareous, or calcareous material, or sometimes an albuminous coagulum or a soft purulent substance. The color of the contents may be white, yellowish,

greenish, orange, or brown, and several of these colors may be observed in the contents of a single cyst. In some cases the cysticercus more or less shriveled and commonly with evaginated head may be readily distinguished upon close scrutiny, but generally is to be found only with difficulty in degenerate cysts. The dead cysticercus found in degenerate cysts usually has a bright-white color which makes it more readily apparent when the contents of the cyst happen to be mostly of a contrasting color. In some of the larger degenerate cysts it is noteworthy that the cysticerci found have been no larger than those found in much smaller cysts. For example, the cysticerci found in two degenerate cysts, 10 by 9 and 10 by 7 mm. in diameter, respectively, measured in their shriveled condition 2 mm. in diameter in one case and  $2\frac{1}{2}$  mm. in diameter in the other and thus were somewhat smaller than the shriveled cysticercus from a cyst 5 by 4 mm. in diameter, which measured 3 by 2 mm.

#### DISTRIBUTION IN BODY

The cysts of *Cysticercus ovis* as found in sheep carcasses are usually comparatively few in number and are commonly limited to the heart or diaphragm, though in many such cases if the muscular parts of the carcass are cut into slices additional cysts are brought to view. Not uncommonly cysts may be found in the muscles of mastication and in the tongue. Sometimes they appear superficially on the muscles beneath the skin, sometimes in the panniculus carnosus itself. The abdominal musculature is not uncommonly affected. Degenerate cysts may be found in the lungs, and in this location they can not be distinguished macroscopically from the small degenerate cysts of *C. tenuicollis*. The parasites have been found in a degenerate condition in the wall of the esophagus. Degenerate cysts found in the wall of the rumen and fourth stomach in a lamb which had been fed segments of tapeworm (pp. 23 and 24) were probably *C. ovis*. Morot has found degenerate cysts in the kidney which may have been *C. ovis*. Degenerate cysticerci in the liver are probably not *C. ovis*, but are more likely *C. tenuicollis*, which frequently occurs in this location. In the writer's experiments none of several lambs fed segments of the tapeworm stage of *C. ovis* showed any invasion of the liver, whereas the liver was affected in each of two lambs fed segments of *Taenia hydatigena*.

*Cysticercus ovis* is therefore essentially a parasite of the intermuscular connective tissue and occurs but rarely in other locations. Except the heart and diaphragm, the parasite appears to have no distinct preference for any particular location in the carcass, and the parts named may appear to be preferred by the parasite simply because these parts are the most readily examined in post-mortem inspection, so that carcasses which have these parts affected are likely to be picked out by inspection, whereas other carcasses which may harbor cysts somewhere in the depths of the musculature are passed by because they show no cysts in accessible parts. The muscles of the head, particularly the muscles of mastication, are

frequently the seat of infestation, and these muscles may be considered as perhaps a preferred location, though this is uncertain. That the tongue is a common location has been established by Dr. W. J. Stewart of the Bureau of Animal Industry, who has found that about one-half of 1 per cent of the tongues of all sheep slaughtered at his station are infested.

#### LOCATION IN SHEEP CARCASSES EXAMINED IN UNITED STATES

In the cases given in Table I the carcasses were examined by slicing the musculature. The number of cysts found in various locations is given. The number found in the head in some instances includes cysts found in the tongue. The columns designated "Superficial" and "Deep" refer, respectively, to cysts elsewhere than in the heart, diaphragm, and head which were either found on a superficial examination of the dressed carcass (Superficial) or were embedded in muscle so that they were found only on dissection (Deep). Cases Nos. 1 to 6 were examined by Dr. I. C. Mattatall at National Stock Yards, Ill.; Nos. 7 to 12 and 13 to 16 by the writer at Seattle, Wash., and Portland, Oreg., respectively; Nos. 17 and 18 by Dr. R. E. Holm at Wallace, Idaho; No. 19 by Dr. E. C. Joss at Tacoma, Wash.; Nos. 20 to 25 by Dr. E. C. Joss at Seattle, Wash.; Nos. 26 to 32 by Dr. E. C. Joss at Portland, Oreg.; Nos. 33 to 35 by the writer at Chicago, Ill.; Nos. 36 to 38 by Dr. I. C. Mattatall at National Stock Yards, Ill.; and No. 39 is lamb No. 1 in the experiments already reported in this article (pp. 23 and 24).

TABLE I.—Location of *Cysticercus ovis* in sheep carcasses examined after dissection.

Case No.	Location of cysts.					Case No.	Location of cysts.				
	Heart.	Diaphragm.	Head.	Superficial.	Deep.		Heart.	Diaphragm.	Head.	Superficial.	Deep.
1.....	1	.....	1	.....	9	21.....	1 or 2	.....	.....	.....	7
2.....	1	.....	1	.....	.....	22.....	1	.....	.....	.....	.....
3.....	.....	1	1	.....	.....	23.....	1 or 2	.....	.....	.....	.....
4.....	2	2	1	.....	8	24.....	1 or 2	.....	.....	.....	.....
5.....	4	1	1	.....	3	25.....	1	.....	.....	.....	5
6.....	14	1	9	.....	30	26.....	1	1	.....	.....	2
7.....	.....	1	.....	.....	.....	27.....	1	.....	.....	2	.....
8.....	.....	.....	.....	1	.....	28.....	.....	.....	.....	3	10
9.....	.....	1	.....	.....	.....	29.....	2	1	.....	.....	3
10.....	.....	1	.....	.....	.....	30.....	.....	1	1	.....	1
11.....	.....	1	.....	.....	.....	31.....	1	.....	1	.....	1
12.....	.....	.....	.....	1	.....	32.....	1	.....	.....	1	.....
13.....	1	.....	.....	.....	3	33.....	1 or more.	.....	2	1	7
14.....	1	.....	.....	.....	3	34.....	18	15	27	2	X <sup>1</sup>
15.....	2	.....	.....	.....	.....	35.....	3	3	3	.....	35
16.....	.....	1	.....	.....	6	36.....	2	.....	1	.....	.....
17.....	1	1	.....	.....	.....	37.....	1	.....	.....	1	1
18.....	.....	.....	1	.....	.....	38.....	1	.....	1	1	3
19.....	.....	.....	.....	2	1	39 <sup>2</sup> .....	X <sup>1</sup>	42	X <sup>1</sup>	X <sup>1</sup>	X <sup>1</sup>
20.....	1 or 2	.....	.....	.....	10						

<sup>1</sup> X indicates numerous cysts.

<sup>2</sup> This carcass also had degenerate *Cysticercus ovis* in the lungs and wall of esophagus and degenerate cysts in the wall of the rumen and fourth stomach which were probably *C. ovis*.



A carcass examined by Dr. O. B. Hess at Seattle, Wash., not recorded above, showed 1 cyst in the heart, 3 in the masseter muscles, 15 in the forequarters, 22 in the "rack," 13 in the saddle, and 7 in one hind leg. The number in the diaphragm or visible superficially was not stated.

Besides the carcasses referred to above there were examined in Chicago in April, 1912, by Dr. W. C. Siegmund and the writer, 59 carcasses which had been retained in the course of routine inspection on account of the presence of cyst in the heart. The examination consisted in examining carefully the diaphragm and the surface of other exposed muscles, examining the internal and external muscles of mastication and tongue after slicing them, and finally examining the cut surfaces after the carcass had been cut into three to five market cuts.

Four carcasses for which the number of cysts in the heart was not recorded showed no additional cysts. Fifty carcasses had one cyst in the heart. Ten of these had additional cysts, three having one cyst each in the diaphragm, two having one and two cysts, respectively, in the muscles of mastication, two having one superficial cyst each in the abdominal musculature and on the hind leg just below the patella, respectively, three having one cyst each on the cut surface of a hind quarter, "rack," and forequarter, respectively, and one having a cyst in the wall of the esophagus. Three carcasses which had two cysts in the heart showed no additional cysts. Two carcasses which had three cysts in the heart showed no additional cysts.

#### DEGENERATION OF CYSTICERCUS OVIS

The cysticerci observed in the course of the routine post-mortem inspection of sheep are usually more or less degenerated, and are either in a condition of caseation or calcification (Pls. III, fig. *E*, and IV, fig. 1). This does not necessarily indicate that live cysticerci are relatively rare. It may be accounted for in part by the fact that degenerate cysticerci are much more conspicuous than the live parasites and, hence, less likely to be overlooked. On the other hand, the validity of this explanation is somewhat offset by the possibility that the cysticerci remain alive only for a short period compared with the length of time they persist in the degenerated condition, in which event one would expect to find degenerated cysticerci more often than living ones. How soon degeneration may begin or how rapidly it may proceed is uncertain, but it is quite clear that in different instances the process varies considerably in these respects and in its character as well. Degeneration as noted elsewhere may occur before the cysticerci have reached their full development. It is probably often influenced by the presence of bacteria introduced by the parasite itself or carried to the cyst by the blood stream, and bacterial action may perhaps have a great deal to do with the large size commonly attained by the degenerate cysts of *Cysticercus ovis*.

The results of the experiments described in another part of this paper prove that degeneration may begin in less than three months after infection, but no data are at hand to show how soon the process may be completed; nor, on the other hand, is it known how long the cysticercus may remain in the tissues of its host before it dies and degenerates.

The various degenerative processes occurring in *Cysticercus ovis* have not been worked out in detail and, hence, will not be considered at length. They are quite similar, at least in some of their variations, to the processes of degeneration which affect *C. bovis* and *C. cellulosae*. A very common occurrence in the case of *C. ovis*, as already alluded to, which seems to be quite unusual in the case of the other two species, is the tendency of degenerate cysts to reach a size which is very large in comparison with the cysticercus itself. In some instances it appears that the increase in size of the cyst may go on indefinitely, fresh calcareous material being continually deposited in the cyst, associated with a breaking down of the inner layers of the capsule and a new growth peripherally.

Like the beef cysticercus, *Cysticercus ovis* tends to degenerate comparatively early when located in the heart. For example, the cysts in the heart of a lamb killed 83 days after infestation (p. 24), so far as observed, were all degenerate. Some of the cysticerci in other locations, including the muscles of mastication, were degenerate, but the great majority were alive. Except in the case of the heart, no definite relation has been observed between the location of the cysticerci and the liability to early degeneration.

The association of live and degenerate cysticerci in the same carcass is a matter of interest, though of less practical importance than in the case of beef and pork measles. In beef measles the association of live and degenerate cysticerci in the same carcass is fairly common. It is often stated in regard to *Cysticercus cellulosae* that if any of the parasites in an infested carcass are degenerated it is likely that all of those present will also be in the same condition. This is by no means invariably true. In a case of pork measles seen by the writer in October, 1912, at an abattoir in Chicago, most of the cysticerci were undegenerated, but degenerate cysticerci were quite common, particularly in the diaphragm and superficial muscles. In the case of *C. ovis*, so far as the writer's experience goes, if the cysticerci found in the heart, diaphragm, muscles of mastication, and other parts of the carcass readily accessible for examination are degenerated, the cysticerci in other parts of the body are likewise, as a rule, in a similar condition. Nevertheless, if *C. ovis* were transmissible to man, it would be unsafe, when only degenerated cysts are found on inspection, to pass a carcass for food upon the assumption that any that might be present in uninspected portions of the musculature would also be degenerated. Live and degenerated cysticerci occasionally, at least, occur together in the same carcass. As noted

above, a considerable number of degenerated cysticerci were found in a sheep 83 days after infection, though most of the parasites were still alive and undegenerated. One other case is recalled in which degenerated and living cysticerci were associated. In this case the cysticerci in the heart, diaphragm, and muscles of mastication were degenerated and partially calcified, as were several found in various portions of the body musculature, but deep in the muscles of one hind leg there was a live cysticercus showing no signs of degeneration whatever.

This accords with what would naturally be expected. One would expect live and degenerate cysticerci in the same carcass as the result, first, of variations in the longevity of cysticerci, as in the case of the experimental sheep mentioned above, or, second, as the result of infestations occurring at different times. It seems that the latter must surely occur often. In view of the close association which commonly exists between sheep and dogs, the sheep in a flock attended by an infested dog are exposed to the chance of repeated infestation, and, hence, sheep must frequently harbor simultaneously cysticerci which have come from eggs ingested on various occasions.

#### DIAGNOSIS OF SHEEP MEASLES

So far as known, the presence of *Cysticercus ovis* can not ordinarily be determined in the living animal, and its diagnosis therefore depends upon a post-mortem examination. It is not always possible to determine definitely whether cysticerci found in sheep or goats are or are not *C. ovis* without resorting to the use of the microscope, but usually microscopic examination is not necessary.

The location of *Cysticercus ovis* in muscle tissue differentiates it clearly from *C. tenuicollis*, which, so far as has yet been proved, is found only in relation with serous membranes. Cases occur, however, in which this rule can not be applied with certainty, as, for example, when the diaphragm or abdominal muscles are involved it is sometimes practically impossible to state on the basis of location alone whether the parasite in question is *C. ovis* or *C. tenuicollis*—that is, the parasite may appear to be in direct relation both with the musculature and the serous membrane which covers the musculature. Here the size of the cysticercus may help to determine its identity; if over 10 mm. (two-fifths of an inch) in diameter, it is *C. tenuicollis*; if less than this size, it is probably *C. ovis*, but may be a young *C. tenuicollis*.

The relation of the head to the caudal bladder—midway between the two ends in *Cysticercus ovis* (Pl. II, fig. 1) and at one end (Pl. II, fig. 5) in *C. tenuicollis*—will indicate the species if the parasite happens to be of a well-marked oval form. Even in very young cysticerci in which the head is yet rudimentary, the relative position of the head is the same as in the fully formed cysticercus. Cysticerci affecting the liver of sheep or

goats may be assumed to be *C. tenuicollis*. *C. ovis* has not as yet been found in the liver. Even in carcasses exhibiting heavy infestation of the musculature, the liver has not been involved. Small-sized cysticerci in the lungs, however, may be *C. ovis*, as degenerate cysticerci of this species have been found in this location in a case of heavy infestation of the carcass.

More difficulty is likely to be experienced in the identification of degenerate cysticerci than of the live parasites, and even more than in the case of the live cysticerci the location must be chiefly depended upon in distinguishing macroscopically between *Cysticercus ovis* and *C. tenuicollis*.

The cysticercal nature of degenerate cysts can often be confirmed by squeezing out the cysticercus, or fragments of it. It should be remembered that the degenerate cyst may be of a much larger size than the contained cysticercus, so that the fact that a cyst is larger than the maximum size of *Cysticercus ovis* does not necessarily exclude this species from consideration. Degenerate cysts of *C. tenuicollis* on the diaphragm or abdominal muscles commonly become more firmly calcified than those of *C. ovis* and show a white, wrinkled surface not seen in the case of the latter.

Excluding from consideration cases of invasion of the musculature by the gid bladder worm, whose true nature will be revealed by examination of the brain and the discovery of characteristic lesions in that location there are two known conditions which may be mistaken for the degenerate cysts of *Cysticercus ovis*: Namely, large *Sarcocystis* nodules and encysted foreign bodies, such as barbs from certain plants which work through the tissues and finally come to rest somewhere in the muscles and become encysted.

In the case of *Sarcocystis* nodules shown in the accompanying illustration (Pl. III, figs. C and D) there were a considerable number of nodules in the diaphragm and heart, 5 mm. and upward in diameter. The walls of these cysts were firm and thick, their contents of a purulent nature. No cysticerci or remains of cysticerci could be discovered. Instead, in each cyst there were found one or more small, transparent vesicles not visible except microscopically. These vesicles, with delicate membranous walls of homogeneous structure without nuclei, contained a finely granular substance and numerous calciform spores about  $15\mu$  long, which demonstrated conclusively that the cysts were *Sarcocystis* cysts. Usually *Sarcocystis* cysts in sheep are so small as to be evident only microscopically, and cysts large enough to be seen with the naked eye are, so far as known, very rare. Knowledge of the characteristics of the unusual forms of *Sarcocystis* cysts such as that described above is too limited to enable one to state definitely the points by which they may be differentiated macroscopically from degenerate *Cysticercus ovis* cysts. In

the case of the latter, however, it is frequently possible by opening the cyst and squeezing out its contents to demonstrate the presence of a cysticercus or the visible and recognizable fragments of one. *Sarcocystis* cysts simulating degenerate *C. ovis* cysts are, so far as appears from our present knowledge, of rare occurrence, and consequently cysts occurring in the musculature of the size and general appearance of degenerate *C. ovis* are presumably *C. ovis* unless there is evidence to show that they are not, such as, for example, the discovery of *Sarcocystis* spores and the total absence of any cysticercus or remnant thereof.

Illustrating the possibility of confusing encysted plant barbules with degenerate *Cysticercus ovis* cysts is a case recently observed in which there was a small nodule about 5 by 4 mm. in diameter in the diaphragm in the muscle tissue just beneath the serosa. This nodule consisted of a thin capsule and contents of a somewhat caseous consistency and might have been taken on casual observation for a small degenerate *C. ovis* cyst. Careful examination, however, failed to reveal any morphological evidence of a cysticercus, instead of which there were found in the midst of the caseous material three or four tiny barbules from some plant, very finely pointed and tapering and spirally coiled. These were scarcely evident to the unaided eye amid the caseous material, but their nature became quite apparent on microscopic examination.

#### GEOGRAPHIC DISTRIBUTION

Abroad, cases of sheep measles have been found in England, France, Germany, Algeria, German Southwest Africa, and New Zealand.

In this country relatively few of the numerous cases found at abattoirs have been traced to the point of origin of the infested sheep. Cases traced to the point of origin have been from Montana (10 counties<sup>1</sup>), Idaho (5 counties<sup>2</sup>), Washington (4 counties<sup>3</sup>), Oregon (11 counties<sup>4</sup>), California (3 counties<sup>5</sup>), Colorado (1 county<sup>6</sup>), and Nevada (middle and western part).

The parasite is probably more or less generally distributed throughout the western United States, and is likely present also in the East, though as yet no cases have been definitely traced to eastern localities. It is probable that it will be found to occur wherever sheep are attended by dogs, particularly wherever dogs have frequent opportunities of devouring dead sheep.

---

<sup>1</sup> Rosebud, Yellowstone, Meagher, Cascade, Choteau, Hill, Blaine, Lewis and Clark, Teton, and Beaverhead Counties.

<sup>2</sup> Fremont, Bonneville, Bingham, Washington, and Canyon Counties.

<sup>3</sup> Adams, Walla Walla, Yakima, and Klickitat Counties.

<sup>4</sup> Polk, Benton, Marion, Multnomah, Crook, Gilliam, Morrow, Umatilla, Union, Wallowa, and Baker Counties.

<sup>5</sup> Modoc, Tehama, and Butte Counties.

<sup>6</sup> Conejos County.

## PREVALENCE

Most of the published records of sheep measles refer to isolated cases found by accident, and accordingly indicate little as to the prevalence of the parasite. Glage (1905), however, in Germany, found degenerate cysticerici in the muscles of 32 out of 2,198 (1.45 per cent) sheep carcasses examined for these parasites by inspection of the head muscles and the heart, and in 16 out of 1,984 (0.8 per cent) in which the heart only was inspected.

Table II shows the total number of sheep slaughtered at 26 meat-inspection stations in the United States during 11 months beginning January, 1912, and the number of carcasses found affected with muscle cysticerici.

TABLE II.—Carcasses of sheep found affected with muscle cysticerici during 11 months at 26 meat-inspection stations in United States.

Station.	Total kill.	Affected.		Station.	Total kill.	Affected.	
	Number.	Number.	Per cent.		Number.	Number.	Per cent.
A .....	898	1	0.01+	O .....	31,237	17	0.05+
B .....	262,361	1	.....	P .....	6,920	1	0.01+
C .....	4,335,153	4,678	.11—	Q .....	116,912	564	.48+
D .....	100,382	34	.....	R .....	19,708	109	.55+
E .....	157,053	12	.01—	S .....	59,759	1	.....
F .....	61,905	107	.17—	T .....	23,381	132	.57—
G .....	55,205	10	.02—	U .....	161,010	1,469	.91+
H .....	237,799	2	.....	V .....	2,106	1	.05—
I .....	1,488,997	2,695	.18+	W .....	1,435,594	5,739	.4 —
J .....	272,739	35	.02—	X .....	526,713	30	.01—
K .....	36,976	23	.06+	Y .....	166,581	19	.01+
L .....	706,584	1,010	.15—	Z .....	86,238	741	.85—
M .....	91,784	1	.....				
N .....	1,429,264	14	.....	Total ..	11,601,898	17,446	.15+

The foregoing table does not indicate accurately the prevalence of sheep measles. In the first place, many cases would necessarily be missed under methods of inspection as nearly perfect as practically possible; in the second place, the methods of inspection for *Cysticercus ovis* have not been developed to the same degree of perfection at the various stations; and finally, at certain stations the methods of inspection for *C. ovis* have reached a high degree of efficiency only in recent months, while the figures given cover also earlier months during which the inspection was less perfect and during which it may even have happened that no cases were found at all. For example, it will be noted from Table II that, in the case of Station R, 0.55 per cent of the sheep slaughtered during January to November were found to be infested. As a matter of fact, however, the great majority of the cases of measles at the station were found toward the close of the period covered; that is, 105 cases, or 2.25 per cent of about 4,300 sheep slaughtered, were found during September, October, and November.

The actual percentage of sheep infested with measles, at least in those sections of this country where a close relationship exists between sheep and dogs, probably approximates 5 per cent much more nearly than it does the very small percentage derived from the figures given in Table II.

#### AGE OF INFESTED SHEEP

Information as to the age at which sheep are most likely to be found infested with measles is meager. A priori it would be expected that rather young animals would most commonly show infestation. As a rule, young animals are more liable to infestation with tissue parasites than old animals, possibly because their tissues offer less resistance to the migration of the parasites than those of older animals. This greater susceptibility is offset to some extent by the fact that the longer an animal lives the more opportunity he has for becoming infested, other things being equal.

Among a total of 189 infested sheep whose ages (approximate) were recorded by inspectors of the Bureau of Animal Industry at several stations, the distribution of cases according to age was as follows:

	Number of cases.		Number of cases.
6 months.....	20	2 to 4 years.....	12
8 months.....	57	3 to 5 years.....	14
10 months.....	3	4 years.....	1
1 year.....	4	5 years.....	10
1½ years.....	3	6 years.....	2
2 years.....	63		

Owing to the lack of data as to the relative numbers of sheep of these various ages which are slaughtered, the figures in the above table do not prove anything. They seem to indicate, however, that *Cysticercus ovis* is more commonly met with in young than in old sheep. As one possible explanation of the apparent rarity of *C. ovis* in old sheep it is reasonable to suppose that as the animals grow older any parasites which they may have picked up in earlier life tend to disappear more or less completely as a result of degeneration and absorption by the surrounding tissues. Meanwhile with increasing age the susceptibility to infestation diminishes, and this, combined with the death and disappearance of the parasites acquired during youth, tends to result in freedom from infestation.

#### ECONOMIC IMPORTANCE

Sheep measles, instead of being as formerly considered a sort of zoological or pathological curiosity, is a matter of great importance to the sheep grower, the butcher, and the consumer of mutton. Although the tapeworm cysts are not transmissible to man, mutton infested with them is not a desirable article of food, and modern ideas in meat inspection require that mutton infested with these parasites to any considerable

extent shall either be condemned or rendered into tallow, according to the degree of infestation, although theoretically there is no objection from the hygienic standpoint to passing affected mutton for food after the parasites have been removed. Practically, however, it is impossible in many cases to remove the parasites, because such extensive dissection would be required that there would be but little left of the meat when the parasites had been removed. Consequently, therefore, a large number of sheep carcasses which are retained by inspectors on account of measles go either to the tallow tank or to the condemned tank, because the character of the infestation is such that it is impracticable to remove the parasites.

At first thought it might seem that the loss on account of these condemnations would fall on the butcher, as the sheep are already bought and paid for before they are passed upon by the meat inspector, but as a matter of fact the producer is made to bear at least a part of the loss. When a condition involving losses on account of condemnations exists among live stock and continues to prevail, the butchers naturally and invariably make ample allowances in the prices paid for the probable loss from condemnations based upon their experience as to losses in the past, so that the producer, although he may not realize it, is made to bear more or less of the burden, sharing it, perhaps, with the consumer, to whom it is likely the butcher will pass on a portion of his loss.

The Federal meat-inspection records, as already noted, indicate that tapeworm cysts in the muscles of sheep are common throughout the West, and furthermore, it is safe to say that the proportionate number of cases of sheep measles found on post-mortem inspection, already representing a high percentage, will continue to increase as meat inspectors become more expert in detecting the presence of the parasites. The natural consequence will be that sooner or later, if this is not already the case, the sheep raiser will suffer a reduction in the selling price of his product below that which he would receive were it not for the losses from condemnations experienced by the butcher.

This indirect loss is in all probability not the only loss experienced by the sheep raiser. It has already been noted that in the experiments five of the lambs died in from 13 to 23 days after infestation. These died approximately in the order of the size of the doses of tapeworm eggs given, those receiving the smallest doses surviving the longest. Three of them received only the eggs contained in a single tapeworm segment, the other two receiving 3 and 10 segments, respectively. The sixth sheep, which survived, received only one-half of a segment, yet the number of eggs was sufficient to make the animal sick for a time, corresponding probably to the period during which the embryonic worms were invading and establishing themselves in the muscles. Quite clearly, therefore, the sheep-measle parasite is deadly in its effects upon sheep, provided a sufficient number of tapeworm eggs are swallowed, and even



if not enough are swallowed to kill the animal, it may be made sick by the invasion of the parasites. Accordingly it is quite probable that many of the cases of death and sickness, which are more or less constantly occurring among sheep without apparent cause, are the result of infestation with the measles parasite.

It has been suggested by Dr. S. W. McClure that sheep measles may be responsible for the many stiff lambs found during spring and summer on the western sheep ranges.

#### SIGNIFICANCE IN MEAT INSPECTION

As *Cysticercus ovis* affects the very part of the carcass which is the most valuable as food—namely, the musculature—it is of great interest in meat inspection and of special importance on account of its prevalence.

Under a system of meat inspection which recognizes but one class of meats as fit for food, such as the system provided for by Federal law in this country, it is proper to pass for food sheep carcasses which show a slight infestation with *Cysticercus ovis* after the removal of the parasites and any lesions caused by them. Carcasses showing more than a slight infestation may be rendered into edible tallow, but if heavily infested should be condemned. As a rule, packers do not take advantage of the provision which permits moderately infested carcasses to be rendered into tallow, but prefer to treat such carcasses the same as condemned carcasses and to manufacture them into inedible products. Though it is possible that all the parasites may not be found and removed from slightly infested carcasses, since it is manifestly impracticable to inspect the depths of the musculature throughout the carcass, it has been determined by experience that there is little likelihood that more than one or two, if any, cysts will be present in the depths of the muscles if only a few are found in the heart, diaphragm, head muscles, tongue, and other superficial or readily accessible parts. Accordingly, if only a limited number of the parasites are found in these locations, there is no reasonable objection to passing such a carcass after their removal.

Even if carcasses are occasionally passed which contain a few cysts that have escaped observation because hidden in the musculature, no great harm is done, since the parasites are not transmissible to man and at most can only offend the esthetic sense of the consumer. Certainly the consequences of passing such carcasses do not balance the great waste which would result if all sheep carcasses infested in any degree whatsoever (amounting to 1, 2, 3, perhaps even 5, per cent of the total number slaughtered) were excluded from use as food. In the light of our present knowledge the German regulations are unnecessarily stringent in placing sheep measles in the same category as pork measles, the basis of these regulations, of course, being the unproved and apparently altogether false assumption that the parasite concerned is *Cysticercus*

*cellulosae*, and hence transmissible to man. Under American regulations concerning *Cysticercus cellulosae*, necessarily more stringent than the German regulations because of the absence of a Freibank in our system of handling meats, no sheep carcass affected with measles even in the slightest degree could be passed for food if the sheep parasite were *Cysticercus cellulosae*. The demonstration of the fact that the muscle cysticercus of sheep is not *Cysticercus cellulosae* and that it is not transmissible to man therefore means that many thousands of sheep carcasses which would otherwise go unnecessarily to the tallow or condemned tank are saved for food, and thus fortunately one of the factors involved in diminishing our already too slender meat supply has been eliminated. Even during the year 1912, when the prevalence of sheep measles was first recognized and before the inspection for *Cysticercus ovis* had been developed to an efficient stage, the money value of sheep carcasses retained on account of measles amounted to nearly \$100,000.

The person who kills mutton for his own use need not be so critical nor so strict with reference to sheep measles as the official meat inspector. The latter, in the absence of legal provision for a Freibank where meat not dangerous to human health but of inferior grade can be sold, has to exclude a great deal of meat from the market which is fit for food under certain conditions, though it can not properly be passed on the same basis as meat unconditionally fit for food. Home-dressed sheep carcasses, therefore, even though infested in a higher degree than would be permitted in carcasses which may pass for food under the Federal meat-inspection regulations may better be utilized for food than wasted, although here the individual will largely be governed by his own feelings in the matter, by his squeamishness or lack of it. Such carcasses, however, should not be sold, at least not without declaration of their nature, as they are obviously of less value than carcasses which are free from infestation.

So far as its detrimental effect on account of the presence of *Cysticercus ovis* is concerned, measly mutton may be eaten with impunity unless the parasites are very numerous or have produced a watery condition or discoloration of the meat, in which case the carcass should be discarded even though the prospective consumer may have no objections to the meat from an esthetic standpoint. In order that further propagation of the parasites may be avoided, a measly sheep carcass discarded from use as human food should not be fed to dogs unless it is first cut into small pieces not exceeding 2 or 3 pounds each and thoroughly boiled.

#### SURVIVAL OF CYSTICERCUS OVIS AFTER DEATH OF HOST

The length of time *Cysticercus ovis* may survive after the death of its host has not been determined. It will, however, live at least six days. Cysticerci in portions of a carcass shipped from Chicago on March 25, 1913, presumably the day of slaughter, and received in Washington on

March 28, were still alive on March 31. After its arrival in Washington the meat was kept in an ice box, at a temperature not lower than 40° F.

As to the period of survival when frozen it was found in one experiment that the cysticerci in a sheep slaughtered on October 15, 1912, were dead on November 7, 23 days after slaughter, the mutton meanwhile having been kept in a frozen condition. Through an oversight no examination of the mutton was made at intervening dates, so that no information was obtained as to how long the parasites actually retained their vitality. The cysticerci were observed by Dr. L. E. Day, who took charge of this experiment on November 7, to be slightly shriveled after thawing. On November 7, about half a pound of the infested mutton was fed to a dog and similar amounts on November 8, 9, 10, and 11. On the last date another dog was also fed. Autopsy on the former dog on December 2, 24 days after feeding, showed no parasites of any kind in the alimentary tract. The other dog when examined post-mortem on January 4, 53 days after feeding, showed a few *Dipylidium caninum*, but no other parasites.

From this experiment it appears probable that a period of three weeks is sufficient, as in the case of *Cysticercus bovis*, to insure the death of cysticerci in mutton. Since, however, *Cysticercus ovis* is not transmissible to man, the same necessity of holding slightly affected carcasses in cold storage for a sufficient period of time to destroy the vitality of any cysticerci which may have been overlooked does not exist. In this respect it is accordingly not so important as in the case of *Cysticercus bovis* to know how long the cysticerci may survive after the slaughter of its host.

#### PROPHYLAXIS

In addition to the highly important preventive measure of destroying the carcasses of all dead sheep by burning, the simplest, most feasible, and most effective means of eradication is to keep the dogs of the ranch or farm free from tapeworms by systematic medicinal treatment. As the sheep-measle tapeworm in dogs begins to produce eggs about two months after infection, judging from the results obtained in the experiments, it is evident that dogs should be treated about every two months in order to remove any tapeworms acquired since the preceding treatment before they have developed sufficiently to produce eggs. In practice, however, such frequent treatment seems scarcely necessary, and it is fairly certain that effective control of tapeworm infestation can be maintained if dogs are submitted to treatment four times a year—that is, every three months. The following method of treatment is employed by Dr. E. T. Davison at the Federal Quarantine Station at Athenia, N. J., and has proved very satisfactory in the case of imported sheep dogs quarantined and treated on account of tapeworm infestation:

Allow the dog to have the usual feed and drink about 3 or 4 p. m. on the day preceding treatment, but give nothing further in the form of food or drink, with

the exceptions noted, until after the medicine has acted. About 10 a. m., to a dog of ordinary size, give four 10-grain capsules filled with ethereal extract of male fern (*Oleoresina aspidii*, U. S. P.), administering at the same time about an ounce of water or milk, preferably the latter. By a 10-grain capsule is meant one which will hold 10 grains of quinine. Forty-five minutes later give a second dose, consisting of four capsules (10-grain) filled with freshly ground areca nut, and with this give as before about an ounce of water or milk. It is important that the areca nut be freshly ground. This treatment is usually followed by profuse defecation and the expulsion of the tapeworm, if any is present, in 30 minutes to an hour after giving the areca nut. No untoward aftereffects have been noted in any case among several hundred dogs treated with this remedy. The patient is usually ready for his evening meal.

In administering the medicine an assistant stands the dog up on his haunches and holds the dog's mouth open by firmly grasping the upper jaw in one hand, the lower jaw in the other. The capsules are dropped on the back portion of the tongue, and enough water or milk is thrown in the animal's mouth to make him swallow. After administering each of the two doses the dog's head should be tied up as high as he can hold it and not choke. If this detail is omitted, the patient will almost invariably throw up the dose. During the remainder of the day the dog should be kept in confinement and the fecal discharges gathered up and burned, buried, or otherwise disposed of in such a manner as to prevent the possibility of contaminating the feed or water of sheep or other live stock.

Incidentally it may be remarked that treating dogs for tapeworm will rid them not only of the sheep-measle tapeworm but also of other species of tapeworm whose intermediate stages are found in live stock, one of which, the gid parasite, fortunately as yet not widespread in the United States, affects the brain of sheep with almost invariably fatal results. Though in certain localities coyotes harboring tapeworms are undoubtedly responsible for some of the infestation of sheep with tapeworm cysts, yet it is the dogs accompanying the sheep more or less constantly day and night and depositing their feces laden with tapeworm eggs in the immediate neighborhood of the sheep which are the chief source of infestation, and if this source is removed by keeping the dogs free from worms, the sheep can be kept practically free from tapeworm cysts of all kinds. In addition, it is important that the carcasses of all dead sheep be destroyed by burning them in order to remove this source of infection of dogs and coyotes.

#### SUMMARY

Sheep measles, or tapeworm cysts in mutton, were first recorded in England in 1866 and the parasite named *Cysticercus ovis* in 1869 by Cobbold. *C. ovis* usually has been considered identical with *Cysticercus cellulosae*, the pork-measle parasite, and also has been confused with *C. tenuicollis*. It has been considered an intermediate stage of a human tapeworm, *Taenia tenella* or *T. solium*, and also of a dog tapeworm, *T. hydatigena* or *T. marginata*.

*Cysticercus ovis* is the intermediate stage of a dog tapeworm, *Taenia ovis* (Cobbold) Ransom. It may attain its full development in sheep in less than three months after infection, and in the dog the tapeworm may

reach the egg-producing maturity in seven weeks after the ingestion of the cysticercus.

*Cysticercus ovis* is commonly limited to the heart or diaphragm, but not infrequently occurs in the muscles of mastication and tongue and sometimes in various locations in the musculature. It may occur in the lungs, the wall of the esophagus, or the wall of the stomach. Doubtful locations are the kidney and liver. It is essentially a parasite of the intermuscular connective tissue and is evidently rare in other locations.

The cysticerci seen by meat inspectors are usually degenerated. Those located in the heart tend to degenerate early. Degeneration may be well established in less than three months after infection. Either partially grown or fully developed cysticerci may degenerate and may be associated with living cysticerci in the same carcass as a result of variations in longevity of the parasites or of repeated infections.

There is no known method of diagnosing the presence of *Cysticercus ovis* in the living animal. The parasites are to be recognised in the sheep carcass by their location in the musculature, by their small size, and by the lateral position of the head of the cysticercus, *C. tenuicollis* being found in relation with serous membranes, being of larger size when fully developed than *C. ovis*, and having its head in an apical position with reference to the caudal bladder. In some cases microscopic examination may be required to differentiate between these two species. The possibility exists of confusing degenerate cysticercus cysts with *Sarcocystis* cysts and with encysted foreign bodies, such as plant barbules.

Sheep measles have been reported from England, France, Germany, Algeria, German Southwest Africa, and New Zealand and have been found in sheep from seven Western States of this country. It probably occurs wherever sheep are attended by dogs, but has not yet been found in sheep known to have originated in the eastern United States (p. 45).

Over 17,000 of the sheep slaughtered under Federal supervision during the year 1912, prior to December 1, were found to be affected with measles. With the development of more efficient methods of inspection for *Cysticercus ovis* the number of cases detected will be relatively much more numerous. The number of infested sheep in the Western States probably exceeds, on the average, 2 per cent of the total number. Young sheep, not over 2 years of age, apparently are more likely to show infestation than old sheep.

*Cysticercus ovis* is of economic importance, first, because of the losses resulting from the condemnation of carcasses found by the meat inspector to be more or less heavily infested, and, second, because of the direct losses which probably occur among sheep as a result of the invasion of the parasites. The extent of these losses can not be estimated at present.

*Cysticercus ovis* is of special interest in meat inspection because it affects the musculature and because it is so prevalent. Carcasses which

are only slightly infested may properly be passed for food after the removal of the parasites, but carcasses showing a heavy infestation should be condemned. Moderately infested carcasses may be rendered into edible tallow, but are usually treated the same as condemned carcasses and are manufactured into fertilizer and other inedible products. As *C. ovis* is not transmissible to man, meat-inspection regulations concerning it need not be so stringent in certain respects as those governing beef measles or pork measles.

The length of time *Cysticercus ovis* may survive after the death of its host has not been determined.

The most important preventive measures against the infestation of sheep with *Cysticercus ovis* consist, first, in destroying by fire the carcasses of dead sheep on the farm or range so that they may not be devoured by dogs or wolves, and, second, in keeping dogs free from tapeworms by systematic medicinal treatment. These measures will also protect sheep from infestation with tapeworm cysts of various other kinds, which they acquire from dogs.

#### BIBLIOGRAPHY

##### ARMBRÜSTER.

1899. *Cysticercus cellulosæ* beim Schaf. Ztschr. f. Fleisch- u. Milchhyg., Berlin, v. 10 (2), Nov., p. 34. [Wa.]  
 1900. *Cysticercus cellulosæ* beim Schafe. [Note.] Ibidem (11), Aug., p. 254. [Wa.]

##### BONGERT.

- 1899 a. Ein Fall von *Cysticercus cellulosæ* in der Muskulatur des Schafes. Ztschr. f. Fleisch- u. Milchhyg., Berlin, v. 9 (5), Feb., p. 86-89, fig. 1-4. [Wa.]

##### CHATIN, JOANNES.

1885. See Railliet, Alcide, 1885 a, p. 234.  
 1886 a. Études sur la laderie du mouton. Mém. Soc. nation. d'agric. de France, Paris, v. 130, p. 191-199. [Wc.]  
 1886 c. Nouvelles recherches sur la laderie du mouton. Bull. Acad. de méd., Paris, v. 50, s. 2, v. 16 (39), 3 oct., p. 242-249. [Wm.]

##### COBBOLD, THOMAS SPENCER.

- 1866 a. Tapeworms (human Entozoa), their sources, nature, and treatment. London, vi+83 p., 15 fig. 12°. [Wa.]  
 1866 r. Specimens of cystic Entozoa from veal and mutton. Tr. Path. Soc. London (1865-66), v. 17, p. 462-463. [MS. dated Apr. 3.] [Wm.]  
 1869 a. Entozoa; being a supplement to the introduction to the study of helminthology. London, viii+124 p., 3 fig. 4°. [Wa.]  
 1873 c. The internal parasites of our domesticated animals; a manual of the Entozoa of the ox, sheep, dog, horse, pig, and cat. London, ix+144 p., 28 fig. 12°. [Wa, Wm.]  
 1873 h. Maddox on an entozoon from the sheep. Lond. M. Rec., v. 1, Aug. 6, p. 487-488. [Wm.]  
 1875 a. Revised list of Entozoa, with notes and references. Veterinarian, London (566), v. 48, s. 4 (242), v. 21, Feb., p. 102-106, (567), s. 4 (243), Mar., p. 169-172. [Wa.]  
 1879 b. Parasites; a treatise on the Entozoa of man and animals, including some account of the Ectozoa. London, xi+508 p., 85 fig. 8°. [Wa.]

## COLBERG.

1900. Ein Fall von Finnen beim Schafe. [Abstract from Verwaltungsber. ü. d. Magdeburg. Schlachthof f. 1898-99.] Ztschr. f. Fleisch- u. Milchhyg., Berlin, v. 10 (4), Jan., p. 71. [Wa, Wm.]

## GILRUTH, J. A.

1908. Report of ... on the Veterinary Division for 1907-8. 16. Rep. Dept. Agric. New Zealand, Wellington, p. 163-214, 3 pl. [Wa.]

## GLAGE, FRIEDRICH.

1905. Beitrag zur Kenntnis der Kalkkonkremente beim Schafe. Ztschr. f. Fleisch- u. Milchhyg., Berlin, v. 15 (7), Apr., p. 204-209. [Wa, Wm.]

## HALL, MAURICE CROWTHER.

1910. A new species of cestode parasite (*Tænia balaniceps*) of the dog and of the lynx, with a note on *Proteocephalus punicus*. Washington, p. 139-151, 9 fig. 8°. [Published Oct. 25.] [Wa.]
1911. Idem. Proc. U. S. Nat. Mus., Washington, D. C. (1780), v. 39, p. 139-151, fig. 1-9. [Wa, Wc, Ws.]
1911. [A case of unusual delay in development of the adult tapeworm, *Multiceps multiceps*.] [Secretary's abstract of note read before Helminthol. Soc., Washington, D. C., Jan. 6.] Science, New York, n. s. (848), v. 33, Mar. 31, p. 510. [Wa.]

## HERTER.

1910. Vorkommen von Rinder- und Schweinefinnen beim Schafe. [Abstract from Landwirtsch. Presse.] Deutsche Schlacht- u. Viehhof-Ztg., Berlin, v. 10 (9), 27. Feb., p. 117. [Wa.]

## KÜCHENMEISTER, [GOTTLOB] FRIEDRICH [HEINRICH].

- 1855 a. Die in und an dem Körper des lebenden Menschen vorkommenden Parasiten. Ein Lehr- und Handbuch der Diagnose und Behandlung der thierischen und pflanzlichen Parasiten des Menschen. Zum Gebrauche für Studierende der Medicin und der Naturwissenschaften, für Lehrer der Zoologie, Botanik, Physiologie, pathologischen Anatomie und für praktische Aerzte. Abt. 1: Die thierischen Parasiten. Leipzig, xvi+486 p., figs., 9 pl. 8°. [Wa, Wm.]

1878. See Küchenmeister and Zürn, 1878-81 a, p. 104.

## KÜCHENMEISTER, [GOTTLOB] FRIEDRICH [HEINRICH]; AND ZÜRN, FRIEDRICH ANTON. [1878-81 a]. Die Parasiten des Menschen. Leipzig, Aufl. 2, x+iv+5-582 p., figs., 15 pl. 8°. [Wa.]

## LEUCKART, KARL GEORG FRIEDRICH RUDOLPH.

- 1856 a. Die Blasenbandwürmer und ihre Entwicklung. Zugleich ein Beitrag zur Kenntniss der *Cysticercus*-Leber. Giessen, 1 p. l., 162 p., 3 pl. 4°. [Wm.]
- 1880 b. Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten. Ein Hand- und Lehrbuch für Naturforscher und Aerzte. Leipzig und Heidelberg, Aufl. 2, v. 1, Lief. 2, Abt. 1, xii+337-856 p., fig. 131-353. 8°. [Wa, Wm.]
- 1886 d. The parasites of man, and the diseases which proceed from them. A textbook for students and practitioners. Natural history of parasites in general. Systematic account of the parasites infesting man. Protozoa-Cestoda. Transl. from the German, with the cooperation of the author, by William E. Hoyle. Edinburgh, xxvi p., 1 l., 771 p., 404 fig. 8°. [Wa, Wm.]

## MADDOX, R. L.

- 1873 a. On an entozoon with ova, found encysted in the muscles of a sheep. [Read before Roy. Micr. Soc., May 7.] Month. Micr. J., London (54), v. 9, June 1, p. 245-253, pl. 18-19. [Wa, Wm, Wc.]

## MONIEZ, R[OMAIN-LOUIS].

1879 c. Note sur le *Tænia krabbei*, espèce nouvelle de *Tænia* armé. Bull. scient. dép. du nord [etc.], Lille, s. 2, v. 2 (5), mai, p. 161-163. [Wm, Wc.]

1880 a. Essai monographique sur les cysticerques. Thèse. Lille, 190 p., 1 l., 3 pl. 4°. [Wm.]

## MOROT, CHARLES.

1897. Observation de nodules intramusculaires de nature indéterminée, a texture fibro-caséuse chez l'agneau et a texture fibro-calcaire chez le cheval. [Read 10 juin.] Bull. Soc. centr. de méd. vét., Paris, v. 51, n. s., v. 15, 30 juin, p. 327-329. [Wa.]

1899 e. Ladrerie et pseudo-ladrerie musculaires chez le mouton. Ibidem, v. 53, n. s., v. 17, 30 déc., p. 495-500. [Wa.]

## OLT.

1898 b. *Cysticercus cellulosæ* in den Muskeln eines Schafes. Deutsche thierärztl. Wehnschr., Karlsruhe, v. 6 (50), 10. Dec., p. 439-440. [Wm.]

## PERRONCITO, EDOARDO.

1900 d. Esiste una *Tænia tenella* diversa dalla *T. solium*? [Read 13 luglio.] Gior. r. Accad. di med. di Torino, an. 63, s. 4., v. 6 (8), agosto, p. 814. [Wm, Wc.]

1900 e. Idem. Gior. r. Soc. ed Accad. vet. ital., Torino, v. 49 (47), 24 nov., p. 1109-1110. [Wa, Wm.]

## RAILLIET, ALCIDE.

1885 a. Éléments de zoologie médicale et agricole. Paris, [Fasc. 1], 800 p., 586 fig. 8°. [Published oct.] [Wa.]

(1902). Cysticercose cardiaque chez un mouton. Bull. Soc. vét. de la Marne, 20 juillet, p. 38.

1904. Idem. [Abstract.] Progrès vét., Agen, an. 17, v. 22 (8), 21 fév., p. 161. [Wa.]

## RAILLIET, ALCIDE; AND MOROT, CHARLES.

1898 a. *Cysticercus tenuicollis* dans la paroi du coeur d'un mouton. [Read 2 avril.] Compt. rend. Soc. de biol., Paris, v. 50, s. 10, v. 5 (13), 8 avril, p. 402-404. [Wa, Wm, Wc.]

## RANSOM, BRAYTON HOWARD.

1908 d. Occurrence of the *Cysticercus* of *Tænia solium* in sheep. [Read before Am. Ass. Adv. Sc.] Science, New York, n. s. (703), v. 27, June 19, p. 950-951. [Wa, Wm, Wc.]

1912. Cysticerci in American sheep, reindeer, and cattle. [Note read before Helminthol. Soc., Washington, D. C., Mar. 14.] Ibidem, n. s. (903), v. 35, Apr. 19, p. 636. [Wa, Wm, Wc.]

1913. An important newly-recognized parasitic disease of sheep. National Wool Grower, Salt Lake City, v. 3 (1), Jan., p. 30-33. [Wa.]

1913. An important newly recognized parasitic disease of sheep. [Secretary's abstract of paper read before 12. Meet. Helminthol. Soc. Washington, D. C., Nov. 21, 1912.] Science, New York, n. s. (941), v. 37, Jan. 10, p. 78. [Wa, Wm, Wc.]

## RICKMANN, W.

1908 a. Tierzucht und Tierkrankheiten in Deutsch-Südwestafrika. Berlin, xi+364 p., fig. A-D. 8°. [Wa.]



## SETTI, ERNESTO.

- 1897 a. Nuovi elminti dell'Eritrea. Boll. mus. di zool. [etc.], Genova (57), 50 p., pl. 8-9, 41 fig. [Wm.]
- 1897 b. Idem. Atti Soc. Ligust. di sc. nat. e geogr., Genova, v. 8 (2), giugno, p. 198-247, pl. 8-9, fig. 1-41. [Wc.]
- 1899 b. Una nuova tenia nel cane (*Tænia brachysoma* n. sp.). Boll. mus. di zool. [etc.], Genova (71), 10 p., pl. 1, 9 fig. [Wm.]
- 1899 c. Idem. Atti Soc. Ligust. di sc. nat. e geogr., Genova, v. 10 (1), mar., p. 11-20, pl. 1, fig. 1-9. [Wc.]

## DESCRIPTION OF PLATES

PLATE II. Fig. 1.—*Cysticercus ovis* from lamb which had been fed eggs of *Taenia ovis* (lamb No. 1, p. 23).

Fig. 2.—*Cysticercus cellulosae*. The cysticerci have been extracted from their cysts. Natural size. (From photographs.)

Fig. 3.—*Taenia ovis*. This tapeworm was developed by feeding *Cysticercus ovis* to a dog (dog No. 6, p. 23). One-half natural size. (From a photograph.)

Fig. 4.—*Taenia hydatigena* (*T. marginata*) from an imported sheep dog.

Fig. 5.—*T. hydatigena* (*T. marginata*) from a dog (dog No. 2, p. 21) which had been fed *Cysticercus tenuicollis*. In figure 5, diagonally above and below, are shown two small specimens of *C. tenuicollis* developed in a lamb (lamb No. 7, p. 25) by feeding segments of *T. hydatigena*. One-half natural size except the two cysticerci, which are shown natural size. (From photographs.)

III (colored). Figs. A and B.—Portions of muscle of sheep showing *Cysticercus ovis* (undegenerated) in situ.

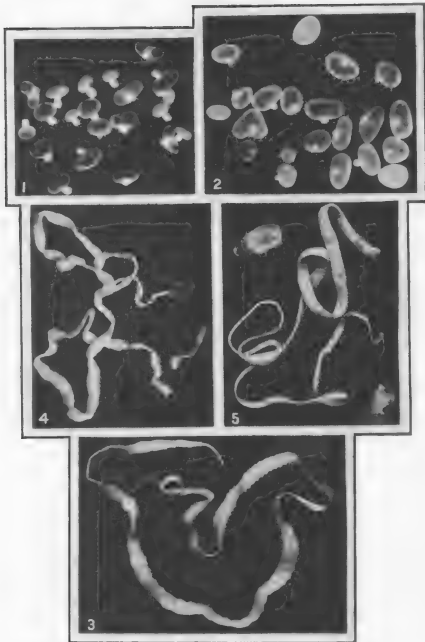
Fig. A.—Section of hind leg showing two "deep" cysticerci. Fig. B.—Hind leg showing three "superficial" cysticerci. (Two-thirds natural size. Original.)

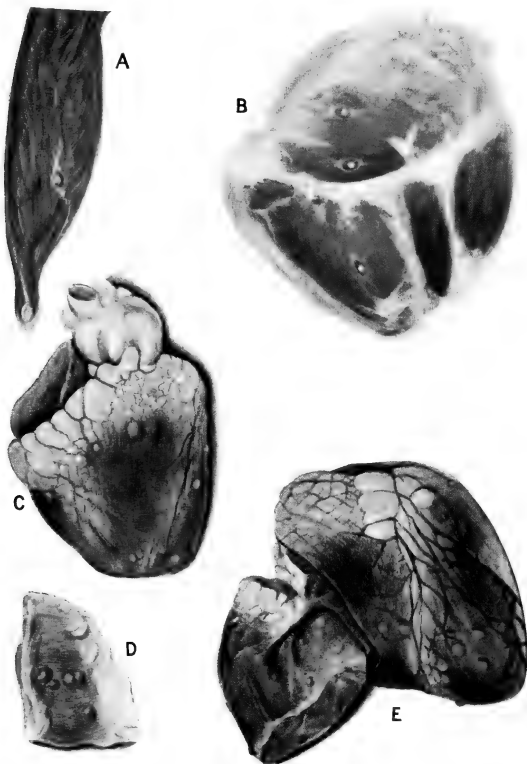
Figs. C and D.—Heart and portion of diaphragm of sheep showing *Sarcocystis* nodules likely to be mistaken for degenerate cysticerci. (Two-thirds natural size. Original.)

Fig. E.—Sheep heart showing numerous small degenerate cysticerci (*Cysticercus ovis*.) (Two-thirds natural size. Original.)

IV. Fig. 1.—Carcass of sheep showing a degenerate cyst of *Cysticercus ovis* at the point indicated by the penknife. (From a photograph by Dr. T. White and Dr. A. English.)

Fig. 2.—Degenerate cysts of *Cysticercus ovis* in muscle of sheep; portion of carcass shown in Plate III, figs. A and B. About natural size. (From a photograph by Dr. T. White and Dr. A. English.)







## THE SERPENTINE LEAF-MINER

By F. M. WEBSTER, *In Charge*, and T. H. PARKS, *Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology*

### INTRODUCTION

The serpentine leaf-miner (*Agromyza pusilla* Meig., fig. 1, a) was described in 1830 from central Europe<sup>1</sup> without definite locality or host plant. The family to which this insect belongs consists of a group of small flies the larvæ of which are largely leaf-miners. Some, however, are known to feed upon scale insects, while *Agromyza tiliae* Couden<sup>2</sup> and *A. magnicornis* Loew<sup>3</sup> are known to make galls on twigs of linden (*Tilia americana*) and on leaves of blue flag (*Iris versicolor*), respectively. Of the species of economic interest in America *Agromyza simplex* Loew occasionally becomes injurious to asparagus<sup>4</sup> by mining the stems. In Australia *A. phaseoli* Coq. seriously injures stems of growing beans,<sup>5</sup> while in India stems of young peas are similarly injured by a species of *Agromyza*.<sup>6</sup>

The habits of *Agromyza pusilla* as a leaf-miner of clovers have long been known, both in Europe and America, and its injuries have been recorded by some of the earliest students of entomology. With the rapid increase of alfalfa culture in the United States, especially in the irrigated sections of the West, the work of this leaf-miner as an enemy of forage crops has been more and more frequently called to the attention of the Bureau of Entomology. During the past three years this insect has been the

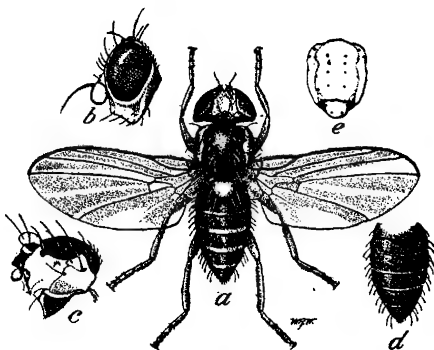


FIG. 1.—The serpentine leaf-miner (*Agromyza pusilla*): a, Adult; b, side view of head; c, side view of thorax, showing characteristic color pattern; d, dorsal view of abdomen, melanic phase; e, outline of thorax, showing location of characteristic bristles. Much enlarged. (Original.)

<sup>1</sup> Meigen, J. W. Systematische Beschreibung der Bekannten Europäischen Zweiflügeligen Insekten. T. 6, Hamm, 1830, p. 185.

<sup>2</sup> Couden, F. D. A gall-maker of the family Agromyzidæ. (*Agromyza tiliae*, n. sp.) Proc. Ent. Soc. Wash., v. 9, p. 34-36, fig. 1, 1907, 1908.

<sup>3</sup> Thompson, M. T. Three galls made by cyclorrhaphous flies. Psyche, v. 14, no. 4, p. 74, fig. 3, Aug., 1907.

<sup>4</sup> Chittenden, F. H. The asparagus miner. (*Agromyza simplex* Loew.) U. S. Dept. Agr., Bur. Ent. Circ. 135, 5 p., 2 fig., 1911.

<sup>5</sup> Froggatt, W. W. The French bean fly. (*Agromyza phaseoli*, Coquillett.) Agr. Gaz. N. S. Wales, v. 22, pt. 2, p. 151-154, Feb., 1911. Also pub. as N. S. Wales Dept. Agr. Misc. Pub. No. 1399.

<sup>6</sup> Maxwell-Lefroy, Harold. Indian Insect Life. Calcutta and Simla, 1909, p. 622-623.

subject of investigations and observations made by several members of the Section of Cereal and Forage Crop Insect Investigations, and the following results are herein set forth regarding this leaf-miner as an enemy of alfalfa (*Medicago sativa*) and other forage crops in America.

#### SYNONYMY

Mr. J. R. Malloch, recently of the Bureau of Entomology, after making a careful study of specimens from Europe and also of a large amount of material from widely separated localities in the United States, includes as synonyms of *Agromyza pusilla* the following names heretofore supposed to apply to valid species:

*A. pusilla* Meig., *A. pumila* Meig., *A. strigata* Meig., *A. exilis* Meig., *A. amoena* Meig., *A. puella* Meig., *A. pusio* Meig., *A. orbona* Meig., *A. blanda* Meig. (?), *A. diminuta* Walker (?), *Oscinis trifolii* Burg., *Oscinis brassicae* Riley.

#### HISTORY OF THE SPECIES IN EUROPE

According to Schiner, "the larvæ mine the leaves of *Euphorbia cyparissias*," the cypress spurge, also called "quacksalver's spurge," which according to Britton and Brown has escaped from gardens to the roadsides and waste places in the Atlantic States.

The same authority quotes Bouché as stating of *Agromyza amoena* Meig. that "the larvæ mine leaves of *Sambucus nigra*, the common European elder."

Kaltenbach records observing the larvæ of *Agromyza trifolii* mining in the leaves of *Trifolium medium* in June and in those of *T. repens* (white clover) in September. He also says of *A. strigata*: "The mining larva lives in leaves of *Campanula trachelium* (bellflower)."

Goureaux,<sup>1</sup> in 1861, records *Agromyza nigripes*, a related European species, as mining in the leaves of *Medicago sativa* (lucerne), in Europe, and his description of the habits and injury caused by these miners is very similar to that which might be given of *A. pusilla* and its injury to alfalfa in America.

Decaux,<sup>2</sup> in 1890, records *A. nigripes* as mining the leaves of lucern in France, and in the infested area estimates a loss of from 20 to 25 per cent of the crop due to the injury to the lucern leaves by this miner.

Groult,<sup>3</sup> in writing of *A. nigripes* in France, records the mines during August and September in fields of lucern and states that where large numbers of the mines were present the devastation became noticeable and the injured lucern made poor forage.

<sup>1</sup> Goureaux, Charles. Les insectes nuisibles aux arbres fruitiers, aux plantes potagères, aux céréales et aux plantes fourragères. Bul. Soc. Sci. Hist. et Nat. de l'Yonne, v. 15, p. 76-454, juill., 1861. "*Agromyza nigripes*," p. 385-386.

<sup>2</sup> Decaux, François. [*Agromyza nigripes* Meig.] Ann. Soc. Ent. France, ser. 6, t. 10 [Bul.], p. ccvii, nov. 26, 1890.

<sup>3</sup> Groult, Paul. L'*Agromyza nigripes*. Le Naturaliste [Paris], an. 30 (ser. 2, an. 22), no. 517, p. 219-220, sept. 15, 1908.

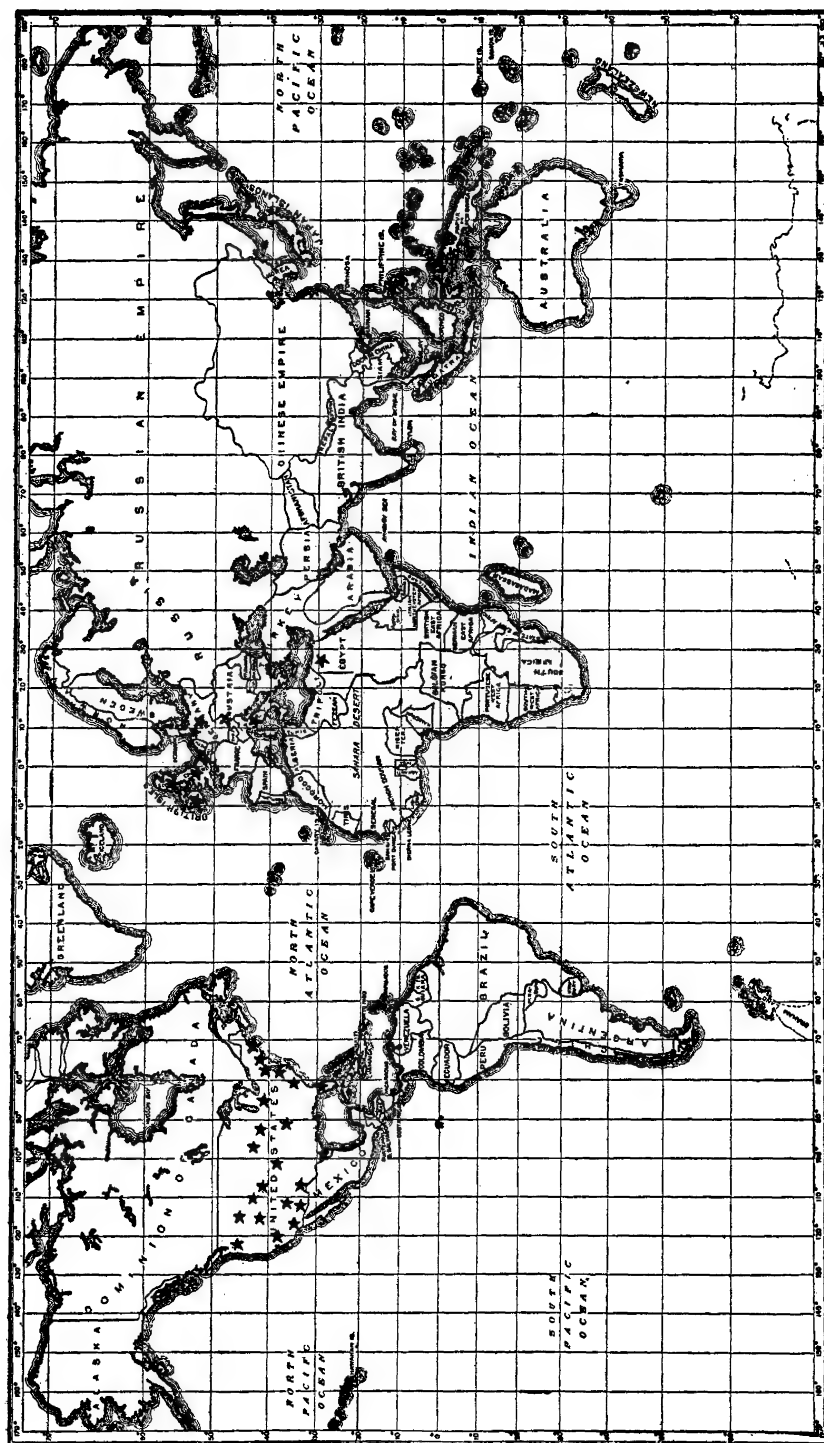


FIG. 2.—Map showing known distribution of the serpentine leaf-miner throughout the world.



Mr. H. S. Smith, formerly of the Bureau of Entomology, noticed dipterous larvæ mining leaves of lucern in fields in Sicily, Italy, and France during the spring of 1912, and from a pupa taken in one of these mines, collected in Sicily during the last week of December, 1911, reared *Agromyza nigripes*. He reports the work of this species in Europe as similar to that of the alfalfa leaf-miner in America with which he is familiar. Apparently the larva can be found mining in the lucern leaves in the latitude of Sicily during the entire winter.

#### DISTRIBUTION OUTSIDE OF THE UNITED STATES

Outside of the United States this species has been found in middle, central, and northern Europe—Italy, Sicily, Egypt, England, Scotland, and Ireland. Its general distribution is shown in the map of the world (fig. 2).

#### DISTRIBUTION WITHIN THE UNITED STATES

The general distribution of the species in the United States, excluding

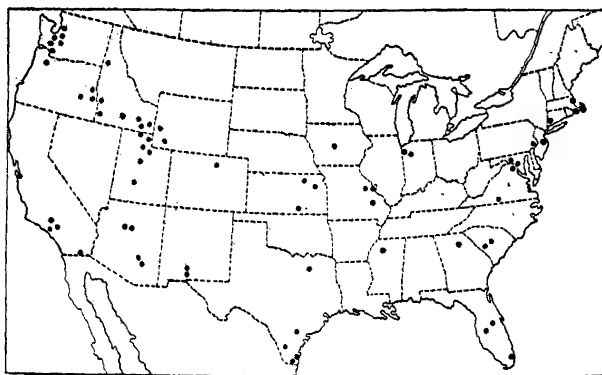


FIG. 3.—Map showing distribution of the serpentine leaf-miner within the United States.

Alaska and the insular possessions, extends from the coast region of central New Jersey southward to southern Florida and westward to southern California and northwestern Washington. It also occurs about Honolulu, Hawaiian Islands. (See map of the United States, fig. 3.)

Specimens are in the collection of the United States National Museum from the following localities:

Washington, D. C. (Coquillett and Pergande); Foristell, Mo. (Riley); Los Angeles, Cal. (Coquillett); Las Cruces, N. Mex.; Douglas County, Kans.; Flagstaff, Ariz.; Williams, Ariz. (H. S. Barber); Honolulu, H. I.; Iowa; Whittier, Cal. (P. H. Timberlake); Biscayne Bay, Fla.; Texas (Belfrage); Plano, Tex. (E. S. Tucker); Cotulla, Tex. (F. C. Pratt); Victoria, Tex. (Hunter).

Specimens in other collections are from the following localities:

Ocean County, N. J. (Dr. John B. Smith); Portland, Oreg. (Melander); Moscow Mt. (Melander); Mt. Constitution, Winlock, Port Gamble, Woodland, Palouse, Monroe, and Olga, Wash. (Melander); Pullman, Wash. (Melander and Hyslop); Philadelphia, Pa. (Henry Kraemer); Danbury, Conn.; Blue Hills, Woods Hole, Auburndale, and Chatham, Mass. (C. W. Johnson).

## FOOD PLANTS IN EUROPE

According to Brischke, Brauer, and Kaltenbach the following host plants in Europe are given for *Agromyza pusilla* and its synonyms:

*Agromyza pusilla* Meig.:

*Spiraea ulmaria* (meadow queen).  
*Solanum tuberosum* (potato).  
*Hyoscyamus niger* (henbane, hog bean).  
*Galeopsis tetrahit* (hemp nettle).  
*Stachys sylvanica* (hedge nettle).  
*Euphorbia cyparissias* (cypress spurge).

*Agromyza strigata* Meig.:

*Campanula trachelium* (bellflower).  
*Taraxacum geniculata* (dandelion).  
*Sonchus oleraceus* (sow thistle).

*Agromyza strigata* Meig.—Continued.

*Bellis perennis* (garden daisy).  
*Agromyza trifolii* Burg.:  
*Trifolium repens* (white clover).  
*Trifolium medium* (zigzag clover).  
*Agromyza orbona* Meig.:  
*Ononis spinosa* (rest-harrow).  
*Ononis repens* (rest-harrow).  
*Agromyza variegata* Meig.:  
*Colutea arborescens* (bladder senna).  
*Agromyza amoena* Meig.:  
*Sambucus nigra* (European elder).

## FOOD PLANTS IN AMERICA

Besides alfalfa, this species has been reared in the United States from the following plants, given here with the locality, date, and collector:

Cabbage (*Brassica oleracea*):

St. Louis, Mo., June 17, 1876 (C. V. Riley); Georgetown, D. C., July, 1882 (Theo. Pergande); Los Angeles, Cal., September, 1887 (D. W. Coquillett); Ames, Iowa, date unknown (Herbert Osborn), reared from stems; Washington, D. C., May and June, 1900 (Theo. Pergande); Athens, Ga., June 7, 1900 (Theo. Pergande); Brownsville, Tex., February, 1908 (D. K. McMillan); Orlando, Fla., March 24, 1908 (H. M. Russell); Honolulu, H. I., September, 1910 (H. O. Marsh), abundant and destructive; La Fayette, Ind., May, 1912 (W. J. Phillips and Philip Luginbill).

## Nasturtium:

Washington, D. C., July, 1897 (D. W. Coquillett); Arlington, Va., June 30, 1906 (I. J. Condit).

Radish (*Raphanus sativus*):

Honolulu, H. I., July, 1906 (Jacob Kotinsky); Washington, D. C., July, 1907 (C. H. Popenoe).

Potato (*Solanum tuberosum*):

Foristell, Mo., June 3, 1876 (C. V. Riley).

Turnip (*Brassica rapa*):

Washington, D. C., July 30, 1906 (I. J. Condit); Corpus Christi, Tex., January 22, 1908 (D. K. McMillan); Arlington, Va., August, 1909 (E. G. Smyth).

Spinach (*Spinacia oleracea*):

San Francisco, Cal., 1907 (E. M. Ehrhorn).

Watermelon (*Citrullus vulgaris*):

Orlando, Fla., June 13, 1907 (H. M. Russell).

Garden beet (*Beta vulgaris*):

Honolulu, H. I., 1906 (Jacob Kotinsky).

Sugar beet (*Beta vulgaris*):

Compton, Cal., April 13, 1910 (H. M. Russell) (adults reared from pupæ collected on leaves); Elsinore, Utah, August 5, 1910 (E. G. Titus) (adults collected on sugar beets).

Pepper (*Capsicum* sp.):

Brownsville, Tex., February, 1909 (D. K. McMillan).

Vetch (*Vicia* sp.):

Columbia, S. C., June 15, 1913 (Philip Luginbill).

Sweet pea (*Lathyrus odoratus*):

Tempe, Ariz., May 24, 1912 (V. L. Wildermuth); Sacaton, Ariz., May 25, 1912 (R. N. Wilson); Salt Lake City, Utah, June, 1911 (C. N. Ainslie).

Fenugreek (*Trigonella foenum-graecum*):

Salt Lake City, Utah, July 22, 1911 (T. H. Parks).

White clover (*Trifolium repens*, Pl. V, fig. 2):

Washington, D. C., June, 1879 (Theo. Pergande); Oxford, Ind., 1884 (F. M. Webster); Washington, D. C., September 11, 1907 (C. N. Ainslie); Salt Lake City, Utah, 1911-12 (C. N. Ainslie and T. H. Parks); Lyman, Wyo., July 14, 1911 (T. H. Parks).

Red clover (*Trifolium pratense*):

Salt Lake City, Utah, June to September, 1911 (T. H. Parks); Twin Falls, Idaho, July, 1912 (T. H. Parks).

Sweet clover (*Melilotus officinalis*):

Tempe, Ariz., May 14, 1912 (V. L. Wildermuth).

Rape (*Brassica napus*, Pl. V, fig. 1):

La Fayette, Ind., 1909 (W. J. Phillips); La Fayette, Ind., 1911 and 1912 (W. J. Phillips and Philip Luginbill).

Cowpea (*Vigna unguiculata*):

Batesburg, S. C., July 12, 1904 (E. G. Titus); Lakeland, Fla., May 8, 1912 (G. G. Ainslie); La Fayette, Ind., July and August, 1912 (Philip Luginbill); Columbia, S. C., July 10, 1908 (G. G. Ainslie), September 11, 1912 (Philip Luginbill); Como, Miss., August, 1912 (T. H. Parks).

Cotton (*Gossypium barbadense*):

Batesburg, S. C., 1912 (E. A. McGregor); Dallas, Tex., 1912 (A. Rutherford).

Tobacco (*Nicotiana* sp.):

Chatham, Va., July, 1906 (W. W. Green).

Hedge mustard (*Sisymbrium officinale*):

Washington, D. C., June, 1900 (F. H. Chittenden and Theo. Pergande); Wellington, Kans., May, 1912 (E. O. G. Kelly).

Smooth rock cress (*Arabis laevigata*):

Washington, D. C., June, 1900 (F. H. Chittenden and Theo. Pergande).

Plantain (*Plantago* sp.):

Salt Lake City, Utah, July, 1912 (C. N. Ainslie).

Common mallow (*Malva rotundifolia*):

Tempe, Ariz., October, 1911 (V. L. Wildermuth).

The great variety in the food plants of the larvæ, together with the fact that the peculiar shaped but rather inconspicuous larval mines in the leaves (Pl. V, figs. 1 and 3) do not readily attract attention except when excessively abundant, leads to the suspicion that the insect may occur unobserved in many localities not indicated on the map (fig. 3). This is perhaps especially true throughout the West wherever it becomes sufficiently abundant in alfalfa fields to be a pest. Therefore, in this paper, it is considered with special reference to alfalfa culture.

## RECORDS OF THE BUREAU OF ENTOMOLOGY

The earliest published record of this insect was by the late Dr. C. V. Riley, who appears to have first reared the fly from larval mines in the lower leaves of potato received from Foristell, Mo., June 3, 1876, other individuals issuing later. At that time it was supposed to be an *Oscinis*.

On June 17, 1876, Dr. Riley noted that cabbage leaves in the vicinity of St. Louis, Mo., were infested by some leaf-mining larvæ, and from these mines a single female fly was reared June 30, the larva pupating underground. Several years later, when apparently the same insect was found mining the leaves of cabbage, June 25, 1882, in Georgetown, D. C., by Mr. Theo. Pergande, interest in Dr. Riley's previous rearing from cabbage leaves in St. Louis, Mo., appears to have been revived. In 1884<sup>1</sup> Dr. Riley described the species as *Oscinis brassicae*, evidently failing to recognize as identical his former rearing from mines in potato leaves, but calling attention to the similarity between his species and *Oscinis trifolii* Burgess, which had been described five years before. This same year (1884) the senior author found the same species in large numbers attacking the leaves of white clover (*Trifolium repens*) at Oxford, Ind.

Three years after its first discovery in Missouri by Dr. Riley and during June, 1879, the insect was observed to be very abundant about Washington, D. C., attacking the leaves of white clover, and was carefully studied by Mr. Theo. Pergande. It must be borne in mind that at that time (1879) it was not positively known to attack clover or other plants elsewhere, and as a result of Mr. Pergande's labors adult flies were secured which were afterwards described by Mr. Edward Burgess as *Oscinis trifolii*.<sup>2</sup>

In 1898 the late Mr. D. W. Coquillett, after examining the types of both *Oscinis brassicae* Riley and *O. trifolii* Burgess, decided that both were synonyms of *Agromyza diminuta* Walk.<sup>3</sup> Further results are shown by Mr. Malloch's studies.

Its wide distribution in the alfalfa-growing section west of the Rocky Mountains was especially noted by the junior author during the summers of 1911 and 1912, when, during the months of June, July, and August, the larvæ were found mining in the leaves of alfalfa at almost every point visited in connection with the investigation of the alfalfa leaf-weevil (*Phytonomus posticus* Gyll.). The territory covered by these observations comprises most of the alfalfa-growing section of Utah, southern and western Idaho, and southwestern Wyoming. In fact, the mines were present in limited numbers wherever alfalfa was found growing and in places widely separated by the uncultivated desert. This may be illustrated by quoting from field notes made at Lucin, Utah, August 20, 1911:

In a small field of alfalfa irrigated from a spring and in the midst of a desert west of Great Salt Lake these leaf-miners were of common occurrence. There is no alfalfa to the east for fully 90 miles and to the west for a distance of about 60 miles, this field being just 6 miles from the Utah-Nevada State line. Both larvæ and pupæ were observed.

<sup>1</sup> Riley, C. V. The cabbage *Oscinis* (*Oscinis brassicae* n. sp.). U. S. Comr. Agr. Rpt. 1884, p. 322, pl. 8, fig. 5.

<sup>2</sup> Riley, C. V. The clover *Oscinis*. (*Oscinis trifolii* Burgess [n. sp.]). U. S. Comr. Agr. Rpt. 1879, p. 200-201, 1880.

<sup>3</sup> Coquillett, D. W. On the habits of the Oscinidæ and Agromyzidæ, reared at the United States Department of Agriculture. U. S. Dept. Agr., Bur. Ent., Bul., n. s., no. 10, p. 78, 1898.

Adults and pupæ were collected at Boise, Idaho, by Mr. H. T. Osborn, of the Bureau of Entomology, August 22, 1911; and from mined leaves of alfalfa received from Sarah A. Armstrong, July 3, 1905, from Fort Collins, Colo., adult flies of this species developed en route.

Its distribution extends westward to the Pacific coast, and throughout the irrigated sections of Washington, Oregon, and California. In a communication dated January 25, 1912, from Mr. Wyatt W. Jones, of Redding, Cal., the writer states that his attention has frequently been called to a minute leaf-miner in alfalfa, very common in that region during August and September. His attempts to rear adults resulted in securing

only parasites. On May 14, 1912, Mr. Jones collected larvæ and pupæ from young alfalfa plants grown from seed sown in March of that year.

Mr. V. L. Wildermuth, who has made a careful study of this insect in the Imperial Valley of southern California and in Arizona, finds it very generally distributed over the alfalfa-growing section of the Southwest, where its injury to the hay crop is probably greatest. It has also been swept from alfalfa at Glendale, Cal., by Mr. T. D. Urbahns.

These flies were reared from larvæ mining alfalfa leaves at Wellington, Kans., by the junior author in July, 1910, and again by Mr. E. O. G. Kelly, of the Bureau of Entomology, at the same place during the summer of 1912. While the injury was not severe, Mr. Kelly reported plants with from 12 to 20 mined leaves common during June.

Two adults and numerous parasites were reared from alfalfa leaves col-

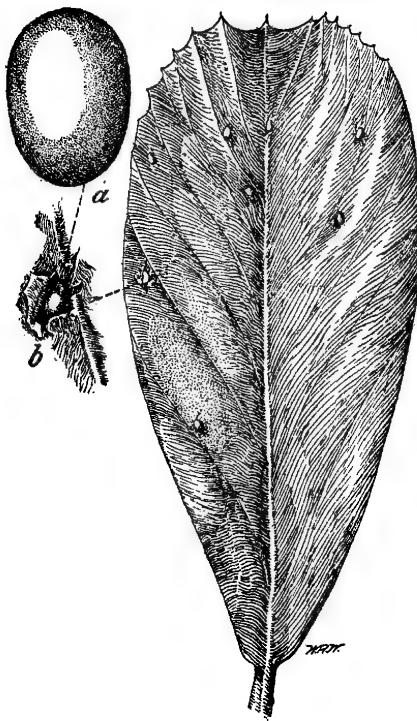


FIG. 4.—Alfalfa leaf with eggs of the serpentine leaf-miner in situ, somewhat enlarged. *a*, Egg, greatly enlarged; *b*, same, in situ, with parenchyma of leaf partly dissected away, much enlarged. (Original.)

lected at Manhattan, Kans., by Mr. C. N. Ainslie in July, 1907. Mr. Ainslie also reared adults and parasites from infested leaves of alfalfa collected at Mesilla Park, N. Mex., May 21, 1909, and reported two or three mines in one leaflet not uncommon in the lower leaves of plants in a field of very young alfalfa.

Specimens have been collected from altitudes varying from below sea level in southern California to 7,000 feet above sea level elsewhere.

Throughout the entire West the mines were found in limited numbers wherever alfalfa is grown.

From the occurrence of the larvæ and pupæ in such widely scattered points we are led to believe that the insect has long been established throughout the alfalfa-growing sections of the West.

While this leaf-miner does not constitute a widespread menace to the alfalfa crop, it works considerable damage in New Mexico, Arizona, and southern California, because leaves mined by the larvæ are unfit for fodder; besides, the changed color of the hay reduces its market value, especially if grown mixed with timothy.

#### DESCRIPTION OF THE LEAF-MINER, *AGROMYZA PUSILLA*.

##### THE ADULT (FIG. 1)

In view of the great number of synonyms and the impossibility of giving descriptions of all of these in this article, Mr. Malloch has drawn up the following description, based on a large number of specimens in the collections of the Bureau of Entomology and the United States National Museum, the better to facilitate the recognition of the insect as it occurs in America.

MALE AND FEMALE.—Black, shining, marked in most variable degree with yellow. Frons, except ocellar region and sometimes a narrow side stripe posteriorly, yellow; remainder of head parts, except behind vertex, yellow. Mesonotum with a more or less broad yellow margin which never extends distinctly around the anterior or the posterior margin; four pairs of dorsocentral bristles present, as well as numerous short hairs on disk; humeri with a black spot. Pleuræ sometimes yellow, with a brownish spot above and shortly behind the coxæ, another large one covering the space between the fore and mid coxæ, and another one between the mid and hind coxæ; at other times almost entirely black, with the sutures and upper margin yellow. Scutellum entirely yellow, or yellow with black basal side spots, which in some cases extend almost around the entire margin and on to the disk. Postnotum black. Abdomen yellowish, with dark brownish bases to segments; or black, with pale apices to segments; or entirely shining black, with the apical segments whitish or yellowish at apex. Legs varying from almost entirely yellow, with only the tarsi brownish, to almost entirely black, with knee joints yellow; the femora generally less intensely black than other parts of legs. Mid tibiæ without distinct posterior bristles. Wings clear; second division of costa about two and one-half times as long as first section, third and fourth veins divergent at extremities; outer cross vein as long as or slightly shorter than the section of fourth anterior to it; basal two sections of fourth subequal or the second slightly the shorter; last section of fifth vein about three times as long as preceding section. Halteres yellow.

Length, 1 to 1.75 mm.

This is a most variable species in color and is very widely distributed.

##### THE EGG (FIG. 4)

The eggs are pale, white, oval, about 0.25 mm. long, and can be frequently seen through the epidermis from above. Figure 4, *b*, shows the egg partly dissected out of one of these pits.

## THE LARVA (FIG. 5)

Larva, newly hatched, about 0.12 mm. in length, nearly white, but soon turning yellowish. When fully developed, it averages nearly 3 mm. in length, fully extended, and is bright translucent yellow, the black, chitinized mouth parts, tracheal system, and dark contents of the posterior alimentary canal being plainly visible through the body walls. Form subacute anteriorly, increasing rapidly in diameter caudad for about one-third of its length, then gradually diminishing posteriorly to the bases of the anal spiracles, where the body becomes rather suddenly truncate, terminating abruptly. Anal spiracles large, porrect, extending beyond end of cauda. Body segments visible and each encircled by a band, granular in appearance, which is sprinkled with minute setaceous tubercles. Anterior spiracles much smaller than posterior, somewhat chitinized at tips, knobbed, and situated in a slight depression.



FIG. 5.—Larva of the serpentine leaf-miner, lateral view. Enlarged. (Original.)

Upon the ventroanal surface there occurs a tubercular, suckerlike organ, in addition to which is a pair of rather large ventrolateral tubercles placed between the anal spiracles and the organ mentioned above. (Description by W. R. Walton.)

## THE PUPARIUM (FIG. 6)

Puparium slightly less than 2 mm. in length, greatest width about 0.8 mm. Oblong oval in form, slightly flattened, the sides sinuate or fluted in outline. Segments strongly marked. Bright yellow in color when freshly pupated, gradually darkening to brown as the development of the pupa progresses. Surfaces slightly shining, but without sculpture. Anterior and posterior spiracles prominent, as shown in figure 6. (Description by W. R. Walton.)

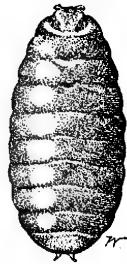


FIG. 6.—Puparium of the serpentine leaf-miner, ventral view. Enlarged. (Original.)

## HIBERNATION

Mr. George G. Ainslie finds that at Lakeland, Fla., the larvæ of the serpentine leaf-miner may continue feeding throughout the entire winter. They were observed by him mining in cowpeas in January, 1913. In the Salt River Valley of Arizona Mr. V. L. Wildermuth finds that during mild winters the larvæ may mine in the leaves until after Christmas. Ordinarily, however, in that locality, the larvæ go into hibernation late in November. At Brownsville, Tex., although we have no information relative to this species, Mr. R. A. Vickery finds that other insects do not hibernate at all, which agrees with what Mr. Ainslie observes to be true of this species in Florida.

It would seem, therefore, that the species hibernates north of Florida and extreme southern Texas and that, so far as known, hibernation takes place only as pupæ on or beneath the surface of the soil. In the North only a small percentage of the last generation in the fall lives to enter hibernation at all, owing to the fact that the larvæ continue feeding in their mines until late in the autumn, large numbers in this way being killed annually by the early freezes of October and November. In the Salt Lake Basin in Utah this insect begins to enter hibernation during

October, although many larvæ continue mining until killed by frosts. Moreover, a very large percentage of the larvæ in the mines are parasitized at this time, which greatly reduces the number of healthy pupæ that would otherwise enter hibernation. The junior author, in an effort to secure hibernating puparia at Salt Lake City in January, 1912, gathered old alfalfa leaves and loose soil from irrigation-ditch banks where the mines had been common during the summer of 1911, but only parasites issued from this material.

Healthy puparia formed late in October pass the winter in that stage in the latitude of northern Indiana.

Hibernation takes place largely in waste places where volunteer alfalfa is found growing. In the arid country of the West such patches of alfalfa can be found everywhere along irrigation-ditch banks, fence rows, and railway right of ways, where it escapes from cultivation.

#### BEGINNING OF ACTIVITY IN SPRING

Adults emerging from hibernation are abroad in April in southern California and Arizona and during the month of May in the intermountain region farther north. Evidently they do not travel far before oviposition takes place. As an indication of this it was noticed, both in Utah and again in Arizona and California, that the first mines observed in spring were usually either confined to the foliage of a single plant or scattered more or less sparingly over two or three adjoining plants. The occupants of these mines, whether larvæ or pupæ, were all in nearly the same stage of their development, thus indicating that the eggs were either deposited by a single female, or, if by more than one, at about the same date. It was noticed, also, that the female confines her oviposition to a small area, usually placing only one egg in a leaf. In the Salt Lake Basin the first mines in spring were usually found clustered on volunteer plants along irrigation-ditch banks, where the insect probably had hibernated.

#### OVIPOSITION AND THE EGG PERIOD

The eggs are deposited in the cellular tissue of the leaf, and the process of oviposition has been observed by several members of the Section of Cereal and Forage Insect Investigations of the Bureau of Entomology. The female deposits the egg from the underside of the leaf, frequently near the margin, where she can anchor herself by hooking the tarsal joints over the edge during oviposition. The fly inserts the ovipositor into the tissues, thrusting the tip of the abdomen against the leaf and puncturing the tissues with her ovipositor. She enlarges the opening thus made by a rotary motion of the abdomen and places the egg well up into the cellular tissue against the epidermis on the upper surface.

After the female has finished enlarging the opening she turns around and sucks up the sap from the aperture, after which she is soon engaged



in making another incision in the leaf, where she repeats the feeding operation. When several females are confined on one plant the under-side of the leaves soon becomes pitted with these feeding punctures made with the ovipositor. Only a small percentage of the punctures contain eggs, as the main function of the punctures seems to be to furnish food for the adults. The larval mine always commences at this little hole or pit.

The females in confinement readily feed on sugar water, and, no doubt, nectar furnishes a part of their food, although no field observations prove this.

The egg period lasts from three to eight days, varying with the seasons of the year, but the average period of incubation can be considered as four days.

#### HABITS OF LARVA AND LENGTH OF LARVAL PERIOD

The larva (fig. 5) commences feeding immediately after hatching and starts mining through the tissues just beneath the upper surface. The



FIG. 7.—Mouth armature of larva of the serpentine leaf-miner, greatly enlarged. (Original.)

mine at first is very small and threadlike, gradually widening with the growth of the larva. Often the miner encircles the entire leaf at first and then works into the uneaten center, and frequently the mine crosses like a figure 8. (See Pl. V, fig. 3.) If the leaf is small, the entire cellular tissue may be consumed, leaving only the epidermis; in such

cases the larvæ have been observed to enter the leaf petioles and burrow a short way downward in an effort to secure enough food to bring them to maturity. The larva is not able to enter a fresh leaf in search of food, but perishes when the food supply in one leaf is insufficient to bring it to maturity.

The larva is provided with an oral appendage, or rasping organ (fig. 7), with which it breaks down the cellular tissue and conveys it to the mouth. This feeding "rake" is swung rapidly from side to side, twice a second or oftener, while the body moves in an arc as far as can be easily reached, when it is quickly brought back to the other end of the "swath" and the body moved up a minute distance to reach new cells. The larva continues thus feeding incessantly within its mine from the time of incubation until maturity. Mr. C. N. Ainslie observed that feeding took place at night as well as by day and that strong transmitted light thrown upon the larva had no effect upon it. It is indifferent to all external happenings, and the epidermis of the leaf may be stripped from the back of the feeding larva without disturbing it, provided the head is not uncovered. When the leaf epidermis is removed from the head, feeding ceases, and the larva can not be induced to resume it.

The larval period covers from 3 to 12 days; during the summer months it is passed in 4 or 5 days, the time increasing as the days get cooler. Many individuals are killed by the autumn frosts while they are yet partially grown. They will, however, continue feeding under remarkably low temperature conditions in an effort to survive; Mr. Wildermuth reared larvæ from the time of hatching till they were full grown in from 10 to 12 days under a mean daily temperature of 46.8° F., and where upon one occasion a minimum of 25° F. was reached.

#### PUPATION AND THE PUPAL PERIOD

The pupa (fig. 6), when found within the leaf, is always at the enlarged end of the mine where the larva stops feeding, and frequently in a cavity next to the lower surface, so that there is no indication that the puparium is present until the leaf is turned over to view it from beneath. The color is light yellow at first and gradually turns darker as transformation progresses, becoming a deep-brown color before the adult emerges. In the more humid section of the country the fully developed larva invariably forsakes its mine and descends into the ground from one-fourth to one-half inch below the surface, or crawls beneath some litter and there pupates. This is apparently true over the entire country with respect to the hibernating generation, but in the arid and semiarid regions of the West it has been observed that during spring and summer much of the transformation takes place within the larval mines in the leaves.

In the Salt Lake Basin and alfalfa-growing sections of southern Idaho and Wyoming pupation occurs almost entirely within the larval mines during the summer months. The junior author, who first studied the species at Wellington, Kans., and afterwards at Salt Lake City, Utah, at once noticed this difference in pupation habits in the two localities. This same thing was noticed at Salt Lake City, Utah, by Mr. C. N. Ainslie, who was rarely able to find mines from which the larvæ had emerged to pupate.

Mr. Wildermuth found that in the Imperial Valley of California during the month of April about 50 per cent of the larvæ pupate in the mines, but in the Salt River Valley of Arizona only a small percentage transforms within the mines, the majority forsaking the leaf and pupating in the soil. In Indiana, where this insect attacks cabbage, rape, and cowpeas, this transformation takes place entirely within the soil. This is also true in the region of the Southeastern States, where the mines are found in the leaves of cowpeas and, as observed by Mr. McGregor, to some extent in those of cotton.

No reason can be advanced to explain this difference in habit of pupation, a careful study of the humidity in these widely separated localities failing to offer any explanation therefor.

The pupal period during the summer months is about 10 days, but ranges from 8 to 28 days from April to December.

### THE ADULT PERIOD

The fly (fig. 1, *a*) emerges through a slit cut in one end of the puparium and can be taken at almost all hours of the day in sweeping the foliage with a net. Adults put in confinement have lived 10 days after emerging, and the time elapsing between emergence and oviposition has varied from 4 to 10 days. The eggs are deposited soon after copulation and in the manner previously described.

### LENGTH OF LIFE CYCLE

The following may be taken as the average period elapsing for the different stages of development during the months of June and July, at a latitude of 40°:

	Days.
Time elapsing between the emergence of the adult and oviposition..	5
Egg period.....	4
Larval period.....	4
Pupal period.....	10
Average time for one generation.....	23

This period is considerably lengthened under existing low temperatures, and a maximum period of 35 or 40 days may be required in the cool weather of late autumn.

### NUMBER OF GENERATIONS ANNUALLY

Since the larvæ continue developing late into the autumn and many of them are killed by the frosts of winter, the number of generations depends entirely upon the latitude, altitude, and length of the growing season. In northern Indiana during the season of 1912 Messrs. Phillips and Luginbill recorded six generations in a series of experiments carried on from the time the first larvæ were found in May until November.

From field observations and generation experiments conducted by the junior author and Mr. E. J. Vosler at Salt Lake City, Utah, there were found to be at least five generations from August 1, 1911, to August 1, 1912. The generation experiments in 1912 were started with adults swept from the fields in May, assumed to have issued from hibernating pupæ. The first generation in the spring is rather well defined and occupies about one month. As the season progresses, the generations so overlap that all stages of the insect can be found in the fields at the same time, and the life cycle was found to be shortened to a minimum of 18 days.

During the latter half of July and the month of August in the Salt Lake Basin it was noticed that the injured leaves of alfalfa in open fields were much more difficult to find than at any other time during the season. Moreover, alfalfa and white clover found growing in the shade were more generally infested than those growing in the open field. This was especially noted at Laketown, Utah, August 4, 1911, where a severe infesta-

tion was noticed on alfalfa plants growing in the bottom of a dry irrigation ditch where the vertical banks on each side kept the plants well shaded. At the same time very few mined leaves could be found in the open fields. There was, however, no interruption to the generation experiments carried on out of doors and in the shade at Salt Lake City, the adults continuing to emerge and larvæ to develop during this time.

Mr. Wildermuth, at Tempe, Ariz., during the season of 1912, remarked the almost total disappearance of all stages during the months of July and August, followed by their reappearance in September. He recorded three generations from the last of April to the last week in June and two more and a partial third generation between September and December of the same year. At Tempe adults did not emerge from the puparia in the generation experiments during July and August.

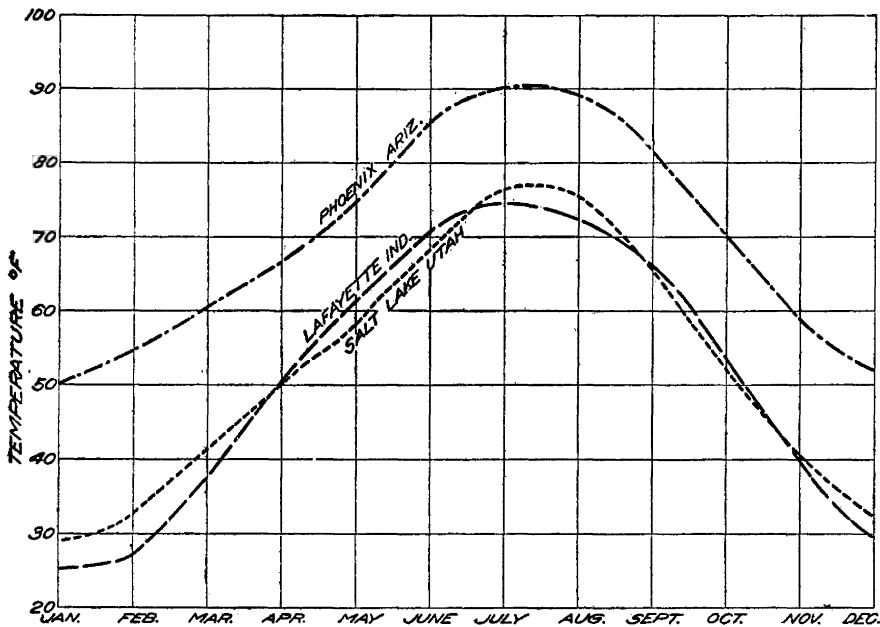


FIG. 8.—Diagram showing the range in temperature throughout the year at three widely separated localities at which observations were made on the serpentine leaf-miner.

In Arizona this disappearance of the insect apparently takes the form of a period of æstivation during the hot weather of midsummer, when the temperature in the open fields is too high for the successful propagation of the species. This is less noticeable in the cooler alfalfa-growing valleys farther north, where the summers are milder. Its presence in Utah alfalfa fields in much reduced numbers during August indicates that an attempt at æstivation is made there, but over a period of much shorter duration than is found farther south, in Arizona.

In this connection we here present (fig. 8) curves representing the normal mean temperatures recorded by the United States Weather Bureau at Salt Lake City, Utah, and Phoenix, Ariz. As will be seen by these curves the normal temperature at Phoenix, Ariz., from the first of

June until early September exceeds the highest mean temperature during the summer at Salt Lake City, Utah. This may in part explain the difference in habits of this insect at the two localities during midsummer.

#### INJURY TO FIELD CROPS OTHER THAN ALFALFA

##### MINING IN LEAVES OF COWPEA

This leaf-miner has been found burrowing in the leaves of the cowpea in widely separated localities by several agents of the Bureau of Entomology.

Dr. E. G. Titus, formerly an agent of the bureau, on July 12, 1904, found the leaves of the cowpea at Batesburg, S. C., generally attacked by leaf-mining larvæ, most of which had already escaped from the mines. He was able to rear two adults of this species and one hymenopterous parasite. Messrs. G. G. Ainslie and Philip Luginbill have observed mined leaves at Columbia, S. C., the former in July, 1908, and the latter in September, 1912. Mr. Luginbill also reared adults and parasites of this insect from their mines in cowpea leaves on the plats of the experiment station at Purdue University, La Fayette, Ind., in connection with studies made at that point extending from July 6 to August 7, 1911. These miners were attended by great numbers of parasitic Hymenoptera, *Euthrichopsis agromyzæ* Vier.

The junior author observed larval mines in cowpeas at several points in Mississippi during August and September, 1912, but in every case the larvæ were parasitized or had escaped from the end of the mine through a slitlike opening and gone into the ground for transformation.

Mr. George G. Ainslie observed considerable injury to the cotyledons of young cowpeas at Lakeland, Fla., May 8, 1912, there being from 2 to 12 mines in each cotyledon—enough to make the leaves appear sickly and white. As many as 10 puparia were secured from moderately infested leaves. The larvæ left the mine to pupate.

The injury to cowpeas is seldom severe, because of the larger size of the leaf, but may become so when the larvæ are present in sufficient numbers in the cotyledon of very young plants before there is sufficient foliage to withstand their attack.

##### MINING IN LEAVES OF RAPE

The larvæ in large numbers were observed by Mr. W. J. Phillips to be mining in rape leaves at La Fayette, Ind., on July 6, 1909, and from the material collected adults of this species emerged July 9. Plate V, figure 1, shows one of these leaves containing several larval mines. The larvæ were observed to leave the mines and pupate on or beneath the surface of the soil, and the complete life cycle was found to be passed in from 25 to 28 days.

More extended studies were made of this species as infesting rape at La Fayette, Ind., during the season of 1912 by Messrs. Phillips and

Luginbill. Mines were also found in leaves of cabbage on May 9. They were first noticed in the leaves of rape on July 12, about the time the mines were noticed in this plant by Mr. Phillips three years before. A series of experiments was carried on from May until November with cabbage and rape as host plants, and a maximum of six generations was found to occur in that latitude.

Here again, as is the case wherever these mines are found, a very large percentage of the larvæ in them were found to be parasitized, and a large number of parasites were reared. Oviposition was observed, both in the field and in confinement, to take place precisely as in the leaves of alfalfa. The mines usually start from near the edge of the leaf, where the eggs are deposited, and extend part way around the leaf on the upper side, being visible only from above.

The extent of the damage to the crop under observation was not severe and, perhaps, could be reduced by destroying all the old plants at the end of the season and plowing deeply in the autumn to bury the hibernating pupæ.

Moreover, since cabbage seems to be a favorite food plant during the spring, it is readily seen that this crop should not be succeeded by or planted near rape, where trouble from this leaf-miner is anticipated.

#### MINING IN LEAVES OF COTTON

While primarily an enemy of forage crops, this miner has been found feeding in leaves of cotton in the Southern States. In 1906, adults were collected in cotton fields at Cotulla, Tex., by the late Mr. F. C. Pratt, and a year later taken in a cotton field by Mr. E. S. Tucker, of the Section of Southern Field-Crop Insect Investigations, Bureau of Entomology. During the summer of 1912, adults determined as this species were reared from cotton leaves at Batesburg, S. C., and Dallas, Tex., by Mr. E. A. McGregor, of the Section of Southern Field-Crop Insect Investigations, and by Mr. A. Rutherford.

The mines were observed at Batesburg by Mr. McGregor from the time of the first appearance of the cotton seedlings until July. Table I, prepared by him, shows the percentage of infestation which existed on July 12, 1912.

TABLE I.—*Infestation of cotton by the serpentine leaf-miner at Batesburg, S. C., July 12, 1912.*

Plants in row.	Plants infested.	Percentage of infestation.
81	69	85
107	84	79
156	136	87
<sup>1</sup> 344	<sup>1</sup> 289	<sup>2</sup> 84

<sup>1</sup> Total.

<sup>2</sup> Average.

Mr. McGregor's notes are as follows:

Data have not been accumulated from which to compute the percentage of leaves affected. It is quite evident, however, that at this season the plants outgrow the infestation and the rapidly forming leaves tend to reduce the percentage of infested leaves. This phenomenon easily leads to the erroneous inference that the pest prefers the seedling leaves and becomes less troublesome as the plants develop. On the contrary, later on in the season freshly formed leaves appear to be just as desirable to the leaf-miner as did the seedling leaves. The tortuous courses of the burrows often sever the main veins of the leaves, causing the death of more or less of the leaf, which may harbor several individuals.

The habits of the leaf-miner, as observed in cotton leaves by Mr. McGregor, are here quoted:

The duration of the larval stage, while not fully established, approximates a week. Feeding takes place and the tunnel is formed in the palisade tissue nearer the upper surface \* \* \*, as the grub increases in size the caliber of the burrow expands until full development is attained at its cavernous end, when the larva escapes through a valvelike incision and pupates in the soil. In the laboratory adults issued six days after pupation.

Three hymenopterous parasites were reared by Mr. Rutherford from the pupæ of the host.

#### NATURAL ENEMIES OF THE SERPENTINE LEAF-MINER

Throughout its entire area of distribution this insect is severely parasitized. Excessive parasitism was noted in the earliest studies of the species about Washington, D. C., and the senior author reared numerous parasites from the larvæ mining in the leaves of white clover at Oxford, Ind., in 1884. In connection with the studies made during the last three years there have been reared at least 28 species of hymenopterous parasites from the mines of this insect in the foliage of alfalfa and other forage crops in the United States. At times these minute enemies have become so numerous as to render even a careful study of the pest itself a matter of some difficulty. But for their presence these leaf-miners would beyond a doubt work much more serious ravages in the alfalfa fields of the West than they do at present. Indeed, one is inclined to wonder what the actual financial effects would be were some condition to arise suddenly whereby the numbers and efficiency of these natural checks were radically diminished.

The first generation of the leaf-miner to appear in the spring is not severely parasitized, and from larvæ and puparia collected at this time numerous flies usually emerge. The following generation is more severely parasitized, and thereafter the parasites increase rapidly, infestation becoming more and more severe, so that mined alfalfa leaves collected during the summer and fall will usually yield parasites instead of adult leaf-miners. To illustrate this point, the junior author, near Salt Lake City, Utah, on September 16, 1911, selected in the field 45 mined alfalfa leaves, 43 of which contained 1 mine each, while 2 had 2 mines. Of the 47 mines,

3 contained healthy larvæ and 2 healthy pupæ of *Agromyza*, while the remaining 42 mines, or 89.7 per cent of those examined, contained parasites. Of these 42 mines, 25 contained parasitized larvæ, 14 parasitized pupæ, and 3 were doubtful. Of the 25 parasitized larvæ, 20 carried 1, and 5 carried 2 external parasites, making 30 parasites on the 25 larvæ of the leaf-miner; these, with the 14 parasitized pupæ, make a total of 44 individual parasites within the 45 mined leaves. In the Salt Lake Basin from June to October, 1911, 75 to 90 per cent of the mines in alfalfa leaves were found to be parasitized.

At Sacaton, Ariz., as early as May 25, 1912, Mr. R. N. Wilson, of the Bureau of Entomology, found 89 per cent of the insects issuing from mines of *Agromyza pusilla* to be parasites, while from material collected there in June and July parasites alone emerged.

Mr. Wildermuth, at Tempe, Ariz., from experiments conducted during the season of 1912, found that much the same degrees of parasitism existed in that locality; and while no record was kept to show the number of parasites found in occupied mines, Table II shows the number of adults and parasites which issued from large numbers of leaves containing *Agromyza* larvæ, collected in the field and kept in jars in the laboratory.

TABLE II.—*Emergence of Agromyza pusilla and its larval parasites in Arizona and California in 1912.*

Date leaves were collected.	Locality.	Experiment No.	Number of <i>Agromyza</i> issued.	Number of parasites issued.	Percentage of parasites to total insects issuing.
May 8	Tempe, Ariz. ....	1	2	80	97
May 10	....do.....	4	4	33	89
May 14	....do.....	6	5	41	89
May 23	....do.....	8	0	68	100
May 31	....do.....	9	3	31	91
June 10	....do.....	10	0	40	100
Sept. 20	....do.....	13	2	12	86
Oct. 1	....do.....	14	3	22	88
Do	....do.....	15	5	24	83
Oct. 14	....do.....	16	3	12	80
Oct. 18	....do.....	17	8	19	70
Oct. 19	....do.....	18	1	12	92
Do	....do.....	19	1	20	95
Do	....do.....	20	9	16	64
Nov. 2	....do.....	21	30	48	61
Total.....			76	478	86.2
Apr. 18	El Centro, Cal. ....		6	18	75
Apr. 20	Brawley, Cal. ....		4	12	75
Apr. 22	Bard, Cal. ....		1	8	88

As will be noted in Table II, the high percentage of parasitism falls off rapidly upon the approach of cool weather, thus enabling the insect to enter hibernation with a much reduced degree of parasitism. At Lakeland, Fla., where no hibernation occurs, Mr. G. G. Ainslie records



no parasites present during January, 1913, among the larvæ feeding in cowpeas. From this fact it naturally follows that the season of greatest injury to forage crops from leaf-miners will be during a period of prolonged cool weather, when the temperature will naturally be unfavorable to the rapid multiplication of the parasites. This is precisely the condition that exists where there are destructive outbreaks of the green bug (*Toxoptera graminum* Rond.) as then the native parasites are unable to keep the pest in check. Of the life history of most of the parasites reared in connection with this leaf-miner comparatively little is known.

**Diaulinus begini** Ashm.—The parasite most thoroughly studied, as well as the most abundant, widely distributed, and hence most important in

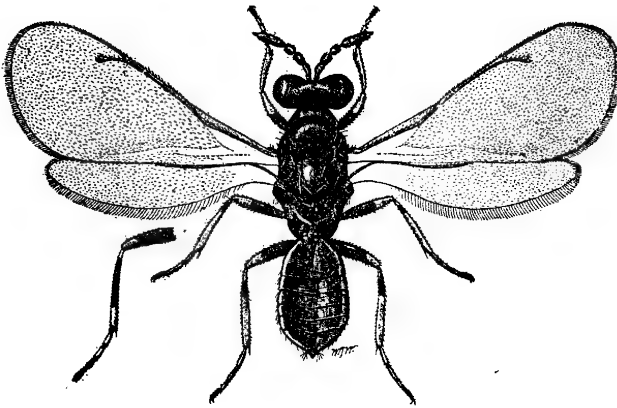


FIG. 9.—*Diaulinus begini*, a parasite of the serpentine leaf-miner. At left, hind leg of *Diaulinus websteri*. Greatly enlarged. (Original.)

the control of the host is a small chalcidoid, *Diaulinus begini* Ashm. (fig. 9), the larva of which feeds externally upon the body of the *Agromyza* larva. This parasite has been reared from mines in leaves of alfalfa, clover, cowpeas, and rape in Indiana, Kansas, Arizona, New Mexico, California, Utah, Wyoming, and Idaho by

different members of the Bureau of Entomology and from mines of *Agromyza parvicornis* in corn leaves at Salt Lake City, Utah.

The junior author was able to observe all stages of its development at Salt Lake City. The female parasite wanders about over the leaf until she locates the *Agromyza* larva in its mine below; then, pushing the ovipositor through the membranous tissue of the leaf which constitutes the roof of the mine, she places the egg upon the body of the host larva. The egg, as observed upon the surface of the host larva, is smooth, translucent, oblong, but rounded about equally at each end, and is about 0.5 mm. in length. The egg period is short, probably not lasting more than one or two days. The young larva feeds externally upon the body of its host, which dies while the parasitic larva is yet very young. Often the presence of the parasitic larva can not be detected on the body of the host without the aid of a microscope. The host larva is invariably dead whenever one of these larvæ, even though apparently just hatched, can be found on its body. Occasionally two larvæ feed on the body of a single host larva, and in one case both parasitic larvæ were observed to complete their transformations and emerge. The larval period is seven days. Figure 10 shows the full-grown larva. Pupation takes place

within the mines of the host and usually some distance away from the remains of its victim. Figure 11 represents the pupa of this species. The pupal period is seven or eight days, and thus the life cycle of the parasite is considerably less than that of the leaf-miner.

***Diaulinus websteri*** Cwfd.\*<sup>1</sup>—*Diaulinus websteri* (fig. 9, a) is very closely related to *D. begini* and, like the latter, it feeds externally upon the larva of its host. In the life-history studies made by the junior author at Salt Lake City, its habits were in no way distinguishable from those of *Diaulinus begini*, the two species being reared together from larvæ found attached to the same host. *Diaulinus websteri* has been reared from *Agromyza* from Kansas, Utah, Arizona, and California, being the most abundant parasite reared in southern California and Arizona. Of the two species of *Diaulinus* reared by Mr. Wildermuth at Tempe, Ariz., this species constituted 66 per cent of the material, while *D. begini* comprised 34 per cent. Of the *Diaulinus* reared at Salt Lake City *D. websteri* comprised only 18 per cent, while 82 per cent were *D. begini*.

This species was reared from mines of *Agromyza pusilla* in hedge mustard at Wellington, Kans., in 1912, by Mr. E. O. G. Kelly. Mr. C. N. Ainslie reared it from mines of *Cerodontha dorsalis* Loew in timothy leaves at Ely, Nev. It is also an enemy of *Agromyza parvicornis* Loew.

***Chrysocharis ainsliei*** Cwfd.\* and ***C. parksi*** Cwfd.\*—These parasites



FIG. 11.—Pupa of *Diaulinus begini*. Greatly enlarged. (Original.)

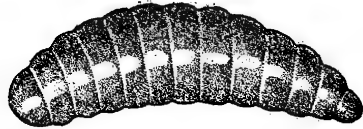


FIG. 10.—Larva of *Diaulinus begini*. Greatly enlarged. (Original.)

(fig. 12) are very important in the control of *Agromyza pusilla* in the West. They feed internally and emerge from the puparia of the host. Their life history is imperfectly known. From hibernation material collected at Salt Lake City during the winter of 1911-12 adults emerged from April 18 to 20, which was 34 days before *Agromyza pusilla* was captured in the fields.

From studies made by the junior author at Salt Lake City, Utah, in 1911 it was noticed that larvæ of *Agromyza* collected in the field, which pupated under observation in the laboratory, would often yield adults of *Chrysocharis* exclusively instead of those of *Agromyza*. Only one parasite issues from each puparium of the host, and dissections made of the puparia often revealed this to be entirely occupied by the larva or pupa of the single parasite, which had entirely consumed its host. But in some instances the puparium of *Agromyza* when dissected revealed two embryo parasitic larvæ within the body of the host larva. As only one adult is known to emerge from each puparium of the host, it is highly probable that when two internal parasitic larvæ

<sup>1</sup> The species of parasites marked with asterisks have been recently described in the Proceedings of the United States National Museum, v. 43, p. 163-188 (1912) by Mr. J. C. Crawford, Associate Curator, Division of Insects.

start to develop in one host, one kills and consumes the other. During September in the Salt Lake Basin 88 per cent of the puparia collected in the mined leaves of alfalfa yielded adults of *Chrysocharis*, and the two species were about equally represented. Both species have been collected in northern and central Utah, southern Idaho, the Imperial Valley of California, and in southern Arizona. *C. parksi* has also been reared from mined alfalfa leaves collected at Redding, Cal., in the Sacramento Valley. It was also reared from *Agromyza* mines in leaves of nasturtium and narrow-leaved plantain at Salt Lake City.

***Derostenus arizonensis* Cwfd.**—This parasite of the larva of *Agromyza* constitutes a new species and is apparently confined to the South-

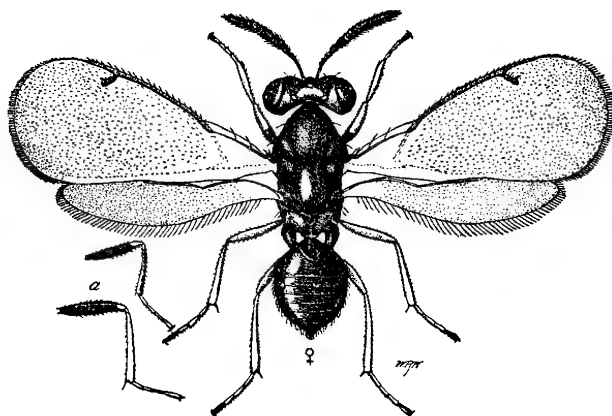


FIG. 12.—*Chrysocharis parksi*, a parasite of the serpentine leaf-miner.  
a, Middle and hind legs of *Chrysocharis ainsliei*. Greatly enlarged.  
(Original.)

west. It was reared in large numbers by Mr. Wildermuth from mined alfalfa leaves collected in the Salt River Valley in Arizona, where it comprised 36 per cent of the larval parasites so reared. Three specimens were reared by Mr. Urbahns from mined alfalfa leaves collected at El Centro, Cal.

A single specimen obtained from the large number of parasites reared at Salt Lake City, Utah, was reared from an *Agromyza* larva in a leaf of fenugreek (*Trigonella foenum-graecum*). It was described by Mr. J. C. Crawford in the Proceedings of the United States National Museum, volume 45, page 315, 1913.

***Derostenus diastatae* How.**—This species has been reared from mines of *Agromyza pusilla* in cowpeas at La Fayette, Ind., by Mr. Philip Luginbill. In the Eastern States it is an important parasite of *Agromyza parvicornis* and *A. angulata*. It has not been recorded west of Kansas.

***Derostenus punctiventris* Cwfd.\***—This insect was reared from puparia of *Agromyza* in mines in leaves of alfalfa at Salt Lake City, by Mr. C. N. Ainslie, and by the junior author, from alfalfa and white clover at Salt Lake City, Utah, and Lyman, Wyo. It was reared only occasionally and is of minor importance as an enemy of this leaf-miner. It also attacks *Agromyza parvicornis*.

***Derostenus pictipes* Cwfd.\***—This parasite was reared from mines of *Agromyza pusilla* in cowpeas at Columbia, S. C., by Mr. G. G. Ainslie in 1908 and at La Fayette, Ind., by Mr. Philip Luginbill in 1911. It was

also reared by Mr. C. N. Ainslie from mines of *A. coquilletti* Malloch in leaves of *Hordeum jubatum* collected at Fort Collins, Colo.

**Derostenus varipes** Cwfd.—A single specimen of this parasite was reared from *Agromyza pusilla* at La Fayette, Ind., by Mr. Luginbill. Nothing is known of its life history. It is a new species and was described by Mr. Crawford in the Proceedings of the United States National Museum, volume 45, page 315, 1913.

**Diaulinopsis callichroma** Cwfd.\*—This species was reared from mines in leaves of cowpea at La Fayette, Ind., by Mr. Luginbill and from alfalfa leaves at Tempe, Ariz., by Mr. Wildermuth. Very few specimens were secured, and it seems of little importance as a parasite of *Agromyza pusilla*.

**Cirrospilus flavoviridis** Cwfd.—Two specimens were reared from mines in alfalfa leaves at Salt Lake City, Utah, by Mr. C. N. Ainslie, who also reared it from mines of *Cerodontha dorsalis* Loew in timothy leaves at Ely, Nev. It is also recorded as a parasite of *Agromyza parvicornis*.

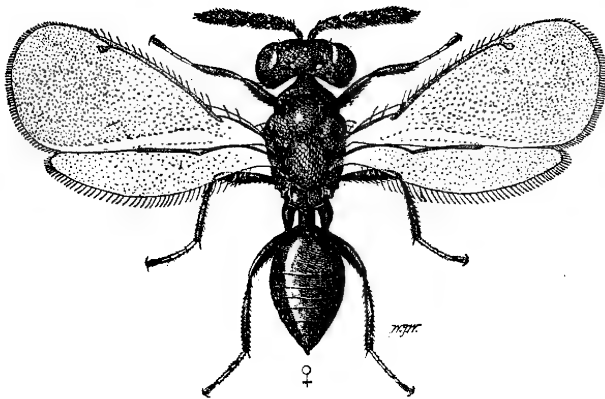


FIG. 14.—*Pleurotropis rugosithorax*, a parasite of the serpentine leaf-miner. Greatly enlarged. (Original.)

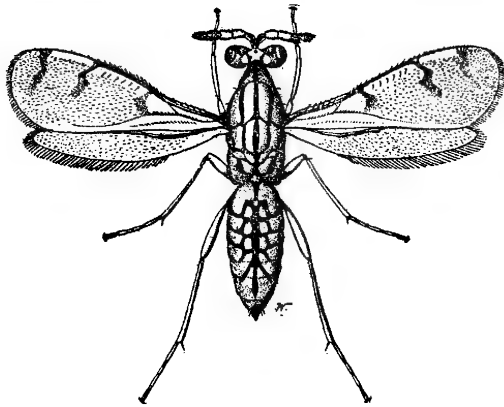


FIG. 13.—*Zagrammosoma multilineata*, a parasite of the serpentine leaf-miner. Greatly enlarged. (Original.)

It was described by Mr. Crawford in the Proceedings of the United States National Museum, volume 45, page 317, 1913.

**Zagrammosoma multilineata** Ashm.—This species (fig. 13), described in 1888, has long been known as a parasite of a lepidopterous leaf-miner (*Lithocolletis* sp.), from which it was reared

by the senior author in Ohio in 1893. Only three specimens were reared from *Agromyza pusilla*, two being reared at Wellington, Kans., by the junior author in 1910 and one by Mr. Luginbill at La Fayette, Ind.

**Closterocerus utahensis** Cwfd.\*—A few specimens of this parasite were reared from mined alfalfa leaves at Salt Lake City, Utah, by Mr.

C. N. Ainslie and at Tempe, Ariz., by Mr. Wildermuth. Nothing is known of its life history. It is also recorded as a parasite of *Agromyza parvicornis*.

**Pleurotropis rugosithorax** Cwfd.\*—This species (fig. 14) was reared sparingly from a puparium of *Agromyza pusilla* by both Mr. C. N. Ainslie and the junior author at Salt Lake City, Utah. It is an internal parasite, having been reared from the immature stages dissected from the puparia of the host. Only one parasite issues from each puparium of *Agromyza*.

**Eucoila hunteri** Cwfd.—This species was not previously known. Two specimens have been reared from puparia of *Agromyza pusilla* by Mr. A. Rutherford at Dallas, Tex. These issued 16 and 17 days, respectively, after the pupation of the host.

**Sympiesis** sp. (?)—One specimen of this species was reared by Mr. Kelly from mines in alfalfa leaves at Wellington, Kans., in 1912. It was also reared from mines in corn leaves at the same locality by the junior author in 1909. This is probably a new species and is not confined to one host.

#### MISCELLANEOUS UNDETERMINED PARASITES

The following miscellaneous Hymenoptera belonging to the superfamily Chalcidoidea<sup>1</sup> were reared from mines of *Agromyza pusilla*, the species being yet undetermined and their life history unknown.

**Pteromalus** sp.—(a) One specimen bearing Webster No. 6639 and reared from mines in alfalfa leaves at Salt Lake City, Utah.

(b) Three specimens bearing Webster No. 7492 and reared at the foregoing locality from mined leaves of white clover.

(c) Two specimens bearing Webster No. 7215 and reared at Tempe, Ariz., from mines in alfalfa leaves.

**Cirrospilus** sp.—One specimen reared from mines in alfalfa at Tempe, Ariz., and bearing Webster No. 7215.

**Diaulinopsis** sp.—Two specimens reared from mines in leaves of cowpea and bearing Webster No. 6395.

**Entedoninae**.—One specimen from mined alfalfa leaves reared at Salt Lake City, Utah, and bearing Webster No. 6639.

#### BRACONID PARASITES

The following species of parasites belonging to the family Braconidae were reared from *Agromyza pusilla* in accordance with the data given below.<sup>2</sup>

**Opius agromyzae** Vier.—La Fayette, Ind. (W. J. Phillips), Nos. 5170 and 6395.

**Opius aridus** Gahan.—Tempe, Ariz., May, 1912 (V. L. Wildermuth), No. 7215.

**Opius brunneipes** Gahan.—Lakeland, Fla. (G. G. Ainslie), No. 9489.

**Opius suturalis** Gahan.—Tempe, Ariz., May, 1912 (V. L. Wildermuth), No. 7215.

<sup>1</sup> Specimens determined to genus or subfamily by Mr. J. C. Crawford.

<sup>2</sup> The determinations are by Mr. A. B. Gahan.

## PREDACEOUS ENEMIES OF THE SERPENTINE LEAF-MINER

Very few predaceous species are known to feed upon the serpentine leaf-miner. This is largely due to the fact that the larvæ feed well concealed within the leaf tissue and are thus not open prey. The following predatory insects are known to feed on some stage of the leaf-miner:

**Triphleps** sp.—These adults are recorded by Mr. E. G. Smyth, recently of the Bureau of Entomology, at Tempe, Ariz., to pierce with their beaks the *Agromyza* larvæ in their burrows.

**Erythraeus** sp.—These red mites are recorded by Mr. Wildermuth at Tempe, Ariz., to attack and kill the *Agromyza* larvæ in their tunnels. Mr. Nathan Banks determines this as probably a new species.

## REMEDIAL AND PREVENTIVE MEASURES

The excessive parasitism under which this species exists has so far prevented it from becoming destructively abundant or doing any widespread serious injury. In case through any cause it should become more injurious to alfalfa, doubtless cutting the crop for hay at once as soon as the depredations were observed would prevent a recurrence. Its greater abundance along ditches, roadsides, and other neglected places indicates that frequent cutting of the alfalfa acts as a permanent check upon the increase of the insect. East of the arid regions deep fall plowing would bury the pupæ so deep in the ground as to put them beyond the possibility of emerging as adults. This is especially recommended for the annuals, such as cowpeas and rape. Throughout the remaining western country keeping down volunteer growth along ditch banks and in waste lands would greatly diminish the number of pupæ which yearly enter hibernation. Of course, pasturing either clover or alfalfa would destroy all larvæ mining in the leaves eaten off by the grazing.

OTHER SPECIES OF THE GENUS *AGROMYZA* LIKELY TO BE MISTAKEN FOR THE SERPENTINE LEAF-MINER

The species of *Agromyza* are for the most part very similar to one another in appearance. As a consequence there has been much confusion in their proper classification, and as a further result of this confusion articles have been published relating to one species which in the light of our present knowledge clearly belong to another. It is with the hope of preventing further errors of this nature that the following species of *Agromyza*—the first of which has in the last year or two been confused with the serpentine leaf-miner—are briefly treated in this paper:

***Agromyza angulata*** Loew.—This leaf-miner (fig. 15) attacks leaves of timothy, mining between the membranes in the same manner as the serpentine leaf-miner.

It was reared from puparia (fig. 16) in leaves of timothy found July 4, 1895, near Bladensburg Road, D. C., by Mr. Theo. Pergande.

During July, 1912, Mr. Philip Luginbill at La Fayette, Ind., reared these adults from mines in leaves of volunteer timothy growing in protected places and was able to secure all stages of the insect.

The eggs are deposited in the cellular tissue just above the epidermis on the ventral side of the leaf, and in punctures similar to those made by *Agromyza pusilla* and *A. parvicornis*.

The egg stage is four to five days.

The larvæ feed in one leaf until mature and pupate in the mine. The larval period is 8 to 10 days; the pupal period, 13 days. This makes a total of 27 days elapsing from egg to adult.

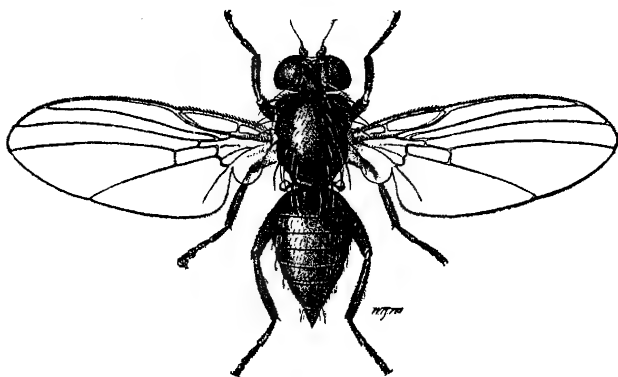


FIG. 15.—*Agromyza angulata*. Greatly enlarged. (Original.)

Mr. Luginbill and Mr. Phillips were also able to transfer these miners from timothy to wheat, rearing one generation from wheat, using as parents flies reared from timothy mines.

The number of generations is not known. The following species of parasites were reared by Mr. Luginbill in connection with his studies in Indiana:

*Polycystus foersteri* Cwfd.; *Derostenus diastatae* How.; *Derostenus agromyzae* Cwfd.; *Pleurotropis rugosithorax* Cwfd.; *Entedon thomsoni* Cwfd.; *Notanisomorpha ainsliei* Cwfd.

A single specimen was collected at Plummers Island, Md., July 28, 1912, by Mr. H. L. Viereck, and specimens collected at Niagara Falls, N. Y., and Auburndale, Mass., are present in the private collection of Mr. C. W. Johnson, curator of the Boston Society of Natural History. The species has never become sufficiently abundant to attract attention.

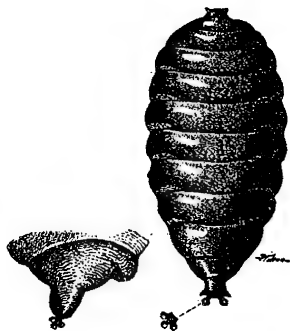


FIG. 16.—Puparium of *Agromyza angulata*, with lateral view of anal appendages at left. Greatly enlarged. (Original.)

***Agromyza coquilletti* Malloch.**—This species (fig. 17) was reared from a puparium found among the basal leaves of volunteer wheat at Bucklin, Kans., November 6, 1909, by Mr. C. N. Ainslie. It was also reared at Fort Collins, Colo., by Mr. Ainslie from a larva mining a leaf of oats, June 30, 1910.

From three larvæ mining leaves of *Hordeum jubatum*<sup>1</sup> in the same locality on July 16, 1910, one adult of this species and seven hymenopterous parasites were reared. These were determined by Mr. J. C. Crawford as *Derostenus pictipes* Cwfd.

Larvæ were observed mining leaves of wheat at Roosevelt, Utah, June 25, 1912, by Mr. C. N. Ainslie, but from this material only parasites of the genus *Pteromalus* issued.

One specimen was reared from a blade of wheat at La Fayette, Ind., July 2, 1912, by Mr. Philip Luginbill, and the junior author reared one adult of this species from a larva mining a leaf of oats taken at Shoshone, Idaho, July 17, 1912.

<sup>1</sup> In this connection we note that Mr. Ainslie reared from various-shaped mines in *Hordeum* collected at Myton, Utah, June 27, 1912, two flies determined by Mr. Walton, of the Bureau of Entomology, as *Hydrellia scapularis* Loew. So far as can be ascertained, this is the first instance of the rearing of this species and the first report that it affects vegetation.

Three specimens have been swept from growing wheat at Manhattan, Kans., by Mr. C. N. Ainslie and one specimen from wheat at Lincoln, Nebr., by Mr. Geo. I. Reeves, of the Bureau of Entomology.

The following localities are represented in the collection of Mr. C. W. Johnson: Twin Rock, Pa. (Johnson); Nantucket, Mass. (J. A. Cushman); Norwich, Vt. (Johnson); Hanover, N. H. (Johnson).

The species has never become a serious enemy of wheat or oats.

**Agromyza virens** Loew.—This species was reared from larvæ taken in root stems of white clover at La Fayette, Ind., by the senior author in August, 1886. The maggots were found singly in the stem, sometimes just under the epidermis, and sometimes in the center. In either case parallel channels were excavated, the larvæ working from the point where the stem originated. These flies were determined tentatively as *Oscinis* sp., and a report<sup>1</sup> of the rearing describing the larva and pupa was published at that time. On October 19, 1898, these flies were reared from larvæ taken in the pith of the garden sunflower (*Helianthus annuus*) at Wooster, Ohio.

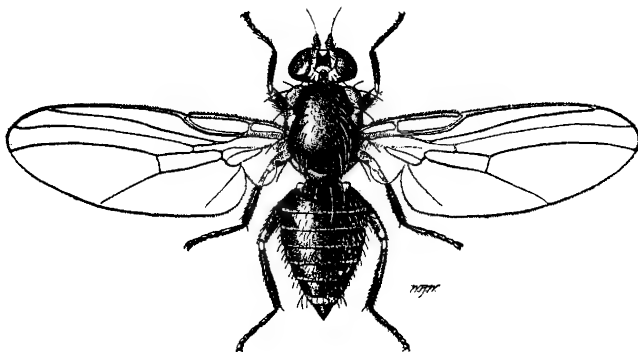


FIG. 17.—*Agromyza coquilletti*. Greatly enlarged. (Original.)

Mr. Theo. Pergande reared adults of this species from stems of *Mulgedium acuminatum* collected by the senior author at La Fayette, Ind., in November, 1885. Several undetermined hymenopterous parasites were reared from this material. These bear No. 3640. Mr. Pergande also reared one adult miner on April 18, 1883, from stems of a weed collected by Mr. Albert Koebele at Holderness, N. H., in October, 1882, and containing at that time mostly pupæ. He also reared an adult from a stem of *Ambrosia artemisiæfolia* (ragweed) received January 6, 1890, from A. M. Sharp at Gladbrook, Iowa.

It has also been reared from heads of *Rudbeckia* sp. at Dallas, Tex.

There are in the collection of the United States National Museum two specimens from Cambridge, Mass., marked "mining in stems of weed" (H. G. Hubbard); two "from stems of Ambrosia," March, 1895, District of Columbia; one "from *Nabalus albus*," May 14, 1883; two from California (Alameda and Los Angeles) collected by Mr. Coquillett; one from Flagstaff, Ariz. (H. S. Barber); thirteen from Toronto, Canada (William Brodie); one from Plummers Island, Md., and four from Washington, D. C.; collected by Mr. W. L. McAtee.

**Agromyza melampyga** Loew, var. **marginalis** Malloch.—Three adults were reared from larvæ mining in leaves of grass (*Paspalum dilatatum*) by Mr. Philip Luginbill at Columbia, S. C., October 4, 1912.

#### SUMMARY

The serpentine leaf-miner is the larva of a minute yellow and black fly which is common in alfalfa fields during the summer.

It is generally distributed over the United States, having a wide range of food plants.

<sup>1</sup> Riley, C. V. The clover-stem maggot (*Oscinis* sp.). U. S. Comr. Agr. Rpt. 1886, p. 582, 1887.



The larvæ injure the foliage of the plant by burrowing between the membranes of the leaf and devouring the parenchyma.

The injury takes the form of a serpentine "mine" which encircles the leaf, gradually widening as the larva increases in size.

Leaves of white clover and frequently of young alfalfa often have the entire cellular tissue devoured, leaving only the two membranes.

There is usually only one larva present in each leaf.

The injury from this insect is greatest in the Southwest, where the discolored leaves, which in severe cases become brown, are sometimes present in sufficient numbers to lower the quality and grade of the hay.

The injured leaves can be found in the fields from May until November, the larvæ continuing to feed until killed by frosts. In Florida the larvæ continue feeding throughout the winter.

The insect hibernates in the puparia beneath the surface of the soil at the base of the plants.

There are five or six generations in latitude  $41^{\circ}$ , the number varying with the length of the growing season.

The generations overlap to such an extent that all stages can be found in the fields during most of the season.

During the period of highest temperature in summer the larvæ are found usually infesting plants protected from the direct rays of the sun. During this period in the arid Southwest the insect almost completely disappears from the fields, reappearing in September.

The eggs are deposited in the leaf tissue and inserted in punctures identical with those made by the adult in feeding. The egg stage during June is 4 days.

The larvæ feed continuously day and night and confine their work to a single leaf. The larval period during June is 4 days.

In the Eastern States pupation occurs entirely in the soil. It takes place commonly in the larval chambers in the leaf in the arid Western States. The pupal period during June is 10 days.

The average period of the complete life cycle is 23 days.

Besides alfalfa the following field crops are subject to attack: Clover, cowpeas, rape, and cotton.

A few nearly related and very similar leaf-miners are known to attack timothy, wheat, oats, and grasses. When these crops are affected, the mine usually extends the entire width of the leaf, and may kill the plant if it is very young.

Numerous parasitic insects attack and consume the larvæ and pupæ within their mines. These are highly efficient and serve to keep the insect in control.

The efficiency of the parasites decreases upon the approach of cool weather.

Many of these parasites are functional in the control of more than one species of leaf-miner, and are very widely distributed.

Frequent cutting of alfalfa kills the larvæ in the leaves and does much to protect this crop. This method should be followed where the injury becomes serious.

Deep fall or winter plowing is advocated for annual forage crops and cereals in order to bury deeply the hibernating puparia located near the surface of the ground.

## BIBLIOGRAPHY

- BOUCHÉ, P. F. Beiträge zur Kenntniss der Insekten-Larven. Stettin. Ent. Ztg., Jahrg. 8, No. 5, p. 142-146, Mai, 1847.  
*"Agromyza amoena* Meig.," p. 142.
- BRAUER, FRIEDRICH. Die Zweiflügler des Kaiserlichen Museums zu Wien. III. Wien, 1883.  
*"Agromyza,"* p. 91-92.
- BRISCHKE, C. G. A. Die Blattminierer in Danzig's Umgebung Schr. Naturf. Gesell. Danzig, n. F., Bd. 5, p. 233-290, 1881.  
*"Agromyza trifolii* Burgess," p. 247; *"Agromyza pusilla* Meig.," p. 249, 270, 273, 274, 288; *"Agromyza strigata* Meig.," p. 260, 266.
- CHITTENDEN, F. H. The native clover leaf-miner (*Agromyza diminuta* Walk.). U. S. Dept. Agr., Bur. Ent., Bul., n. s., no. 33, p. 77, 1902.  
*"Agromyza diminuta* Walk."
- COMSTOCK, J. H. The clover Oscinis (*Oscinis trifolii*, Burgess [n. sp.]). U. S. Comr. Agr. Rpt. 1879, p. 200-201, 1880.  
*"Agromyza (Oscinis) trifolii* Burgess."
- COQUILLET, D. W. On the habits of the Oscinidæ and Agromyzidæ reared at the United States Department of Agriculture. U. S. Dept. Agr., Bur. Ent., Bul., n. s., no. 10, p. 70-79, 1898.  
*"Agromyza diminuta* Walk.," p. 78.
- KALTENBACH, J. H. Die Pflanzenfeinde aus der Klasse der Insekten. Stuttgart, 1874.  
*"Agromyza orbona* Meig.," p. 113; *"Agromyza strigata* Meig.," p. 408; *"Agromyza amoena* Meig.," p. 298-299; *"Agromyza trifolii* Burgess," p. 129.
- LINTNER, J. A. The insects of the clover plant [read Jan. 19, 1881]. Trans. N. Y. State Agr. Soc., v. 33, 1877-1882, p. 187-207, 6 fig., 1884.  
*"Agromyza (Oscinis) trifolii* Burgess," p. 205-206.
- RILEY, C. V. The cabbage Oscinis. (*Oscinis brassicæ*, n. sp.). U. S. Comr. Agr. Rpt., 1884, p. 322, pl. 8, fig. 5.  
*"Agromyza brassica* Riley."
- SCHINER, J. R. Fauna Austriaca. Die Fliegen (Diptera). T. 2, Wien, 1864.  
*"Agromyza pusilla* Meig.," p. 301.

#### DESCRIPTION OF PLATE

PLATE V. Leaves of different species, showing the work of the serpentine leaf-miner (*Agromyza pusilla*). Fig. 1.—Mines in a leaf of rape. Fig. 2.—Mines in leaves of white clover. Fig. 3.—Mines in leaves of alfalfa. (All nearly natural size. Original.)

(88)

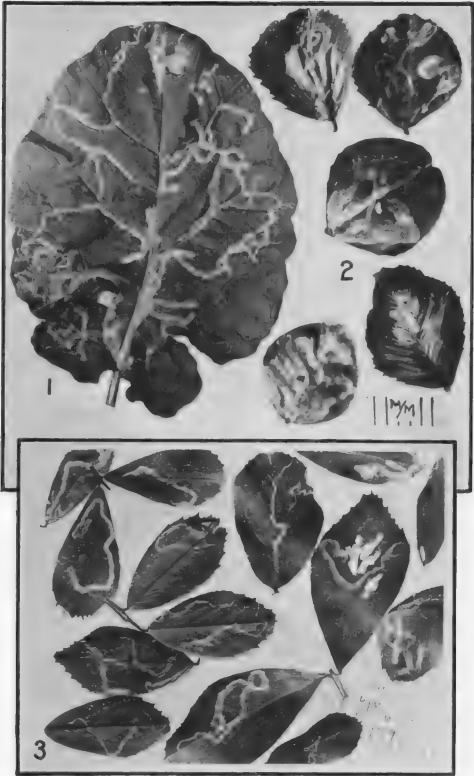
---

---

ADDITIONAL COPIES of this publication  
may be procured from the SUPERINTEND-  
ENT OF DOCUMENTS, Government Printing  
Office, Washington, D. C., at 15 cents per copy

---





# JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

---

VOL. I

WASHINGTON, D. C., NOVEMBER 10, 1913

NO. 2

---

## THE OCCURRENCE OF A COTTON BOLL WEEVIL IN ARIZONA

By W. DWIGHT PIERCE,

*Agent and Expert, Investigations of Insects Affecting Southern Field Crops,  
Bureau of Entomology*

The preliminary announcement by Mr. O. F. Cook, of the Bureau of Plant Industry, in February, 1913, of the occurrence in Arizona of a weevil resembling the Mexican cotton boll weevil, appears at this time to have been an announcement of considerable importance. In company with Mr. Harold Bell Wright, Mr. Cook found this weevil breeding in the bolls of a wild shrub known as *Thurberia thespesioides* in Ventana Canyon, Santa Catalina Mountains, Arizona.

In May the writer obtained a large quantity of bolls of *Thurberia* from Mr. W. B. McCleary, of the Bureau of Plant Industry, who collected them in the lower part of Stone Cabin Canyon, Santa Rita Mountains, Arizona. This material was very heavily infested by the weevil.

During August Dr. A. W. Morrill, State Entomologist of Arizona, together with the writer, located this weevil in Ventana Canyon, Santa Catalina Mountains, and in Sawmill Canyon, Santa Rita Mountains, breeding commonly upon the same plant.

A close examination of the material received early in the year disclosed many minor points of difference from the usual form of the cotton boll weevil, *Anthonomus grandis* Boheman. The Arizona form averages slightly larger and is a little more robust. The punctuation of the male beak is a little more pronounced, and the sculpturing throughout is slightly stronger than in the Texas form. The scaly vestiture approaches a golden color, while in the Texas form it is usually grayish. The sides of the prothorax in front are rarely emarginate, while the emargination is usually very noticeable in the Texas form. Minor differences also appear in the shape of the teeth on the legs. All in all, the adults of the Arizona weevil present an assemblage of characters differing from the eastern form sufficient to suggest a new species.

In addition to these differences in characters, specimens of the Arizona form were found in hibernation in their cells until September 1, while the eastern form is never found in its cells in cotton bolls after March 15. The Arizona insect seems to be confined to one, or not more than two, annual generations, while the cotton boll weevil has many generations. The former lives on *Thurberia*, the latter on *Gossypium*. The Arizona weevil was found at 4,000 feet altitude, while the Texas weevil has never been found above 2,000 feet altitude. The two forms are geographically isolated by mountain divides. When the Arizona weevil was seen in the field, it displayed a tendency to oviposit at a different place and to seal its egg puncture differently; the egg itself was of a slightly different shape.

The Mexican cotton boll weevil has never been known before this year to feed readily or breed in any other plant, although suspected of being capable of adapting itself to other foods if forced to it. When opportunity was given the Texas boll weevil to attack *Thurberia* squares and bolls, it fed readily and eagerly, sometimes displaying a preference for *Thurberia* over cotton when both were available. The *Thurberia*-feeding weevil, on the other hand, was able to feed upon and breed in cotton squares.

Mr. B. R. Coad, of the Bureau of Entomology, has succeeded in rearing undoubted crosses between the two varieties from females of each form, although these hybrid offspring were somewhat undersized.

It will be seen from further evidence in this paper that the two forms must represent merely two subspecies, or varieties, or geographic races of a single species. The Arizona form is therefore to be known as *Anthonomus grandis thurberiae*, new variety. Its technical description is as follows:

***Anthonomus grandis thurberiae*, n. var.**—Stout, subovate, rufo-piceous, and clothed with coarse, pale-yellowish pubescence. Beak long, slender, shining, and sparsely pubescent at the base; striate from base to the middle, striae rather coarsely punctured; apical half finely and remotely punctured. Antennae slender, second joint of funicle longer than the third; joints 3 to 7 equal in length but becoming gradually wider. Head conical, pubescent, coarsely but remotely punctured, front foveate. Eyes moderately convex, posterior margin not free. Prothorax one-half wider than long; base feebly bisinuate, posterior angles rectangular; sides almost straight from base to middle, strongly rounded in front; apex slightly constricted and transversely impressed behind the anterior margin; surface moderately convex, densely and sub-confluently punctured; punctures irregular in size, coarser about the sides; pubescence more dense along the median line and on the sides. Elytra oblong, scarcely wider at the base than the prothorax; sides robust to subparallel for two-thirds of their length, thence gradually narrowed to and separately rounded at the apex, leaving the pygidium moderately exposed; striae deep, punctures large and approximate; interstices convex, rugulose, pubescence somewhat condensed in spots. Legs rather stout, femora clavate, anterior strongly bidentate, inner tooth long and strong, outer one acutely triangular and connected with the former at the base; middle femora with small second tooth and posterior femora unidentate. Tibiae moderately stout, anterior bisinuate internally, posterior straight; tarsi moderate, claws broad, blackish, and

rather widely separate; tooth almost as long as claw. Length, 5 to 5.5 mm. (0.20 to 0.22 inch).

This variety differs from *Anthonomus grandis* on cotton by its greater robustness (Pl. VI); the more golden appearance of the scales; the slighter constriction of the prothorax (figs. 1 and 2); its stouter and more coarsely sculptured beak (figs. 3 and 4); its slightly more compact antennæ (figs. 5 and 6), with funicle of a lighter color than the club; its stouter legs, with a distinct second tooth on the middle femora (figs. 7 and 8); the wing (fig. 9), which shows a slightly more distinct spot. It also differs in its food plant (*Thurberia thesperoides*), its altitude (4,000 feet upward), its breeding season (August 15 to November), and in certain physiological and biological characters. The most obvious diagnostic characters are as follows:

***Anthonomus grandis thurberiae***

Antennal funicle of a distinctly lighter color than the club; punctation of elytral striæ strongly and clearly defined; prothorax usually very feebly constricted and not emarginate or but very slightly so; elytra often robust; vestiture of ochreous scales intermixed with black hairs; breeds in *Thurberia thesperoides*; range, above altitude of 4,000 feet.

***Anthonomus grandis***

Antennal funicle and club concolorous; punctation of elytral striæ not clearly defined from the striæ; prothorax strongly constricted at apex and usually emarginate in front; sides of elytra usually parallel; vestiture of grayish to ochreous scales intermixed with very inconspicuous grayish to very dark-brown hairs; breeds in *Gossypium* spp.; range, below altitude of 2,000 feet.

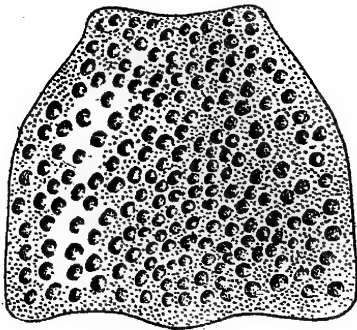


FIG. 1.—*Anthonomus grandis*, var. *thurberiae*: Prothorax. Much enlarged. (Original.)

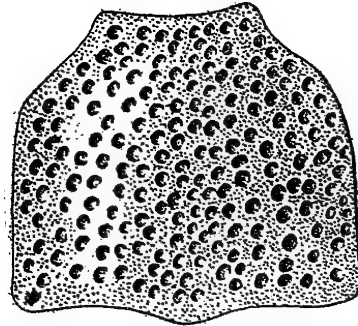


FIG. 2.—*Anthonomus grandis* Boh.: Prothorax. Much enlarged. (Original.)

**HIBERNATION.**—It is not known whether *Anthonomus grandis thurberiae* hibernates as an adult outside of its cell, but it is known positively that many individuals pass the winter and even the summer in the cells formed during the preceding fall. In May, 1913, from the material sent by Mr. McCleary, the writer found 18 live adults in their cells in an examination of 743 bolls, 220 of which were infested. On August 27 Dr. Morrill found six live boll weevils still in their last year's cells at about 4,500 feet altitude in Sawmill Canyon, Santa Rita Mountains, and on August 30 the writer found another live weevil in its cell in Ventana Canyon, Santa Catalina Mountains.

As further evidence of the prolonged rest of this variety, no immature stages were found, beyond a one-fifth grown larva in squares. The extreme lateness of the plants in the canyons where the boll weevil was found indicated that the weevils could not have had buds on which to feed for much more than two weeks in August. Plants grown from seed at Victoria, Tex., and Tallulah, La., did not begin to produce buds until well along in August. The natural dormant period of the Arizona boll weevil therefore lasts about nine months.

It is interesting to note that the *Thurberia* weevils extracted from their cells in May and sent to Victoria, Tex., immediately began to feed and breed upon cotton and produced several generations.

The Arizona form has either acquired by long years of adversity an ability to survive for a longer period without food, assuming *Anthonomus grandis* Boh. to be the original species; or if the *Thurberia* weevil is the true original form, then the ability to obtain a plentiful supply of early food has caused the species to lose some of its resistance to adversity.

**FEEDING.**—The adults feed upon the squares and bolls in much the same manner as the typical *Anthonomus grandis*.

**FEIGNING DEATH.**—The adults are not quite so easily disturbed as those of the cotton-feeding form, but when disturbed they feign death and drop to the ground or fly away.

**OVIPOSITION.**—On the first day that any adults were seen, August 25, in the Santa Rita Mountains, the males were the most abundant and usually were not feeding, but were perched on the tips of squares or on the foliage in an attentive attitude, evidently waiting for females.

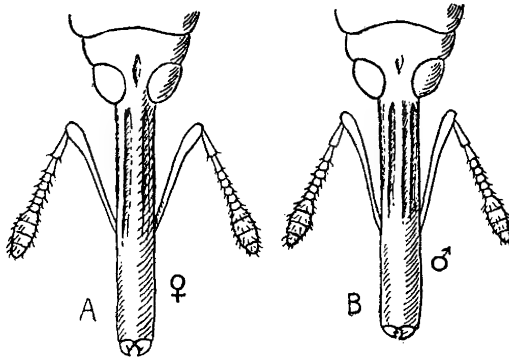


FIG. 3.—*Anthonomus grandis*, var. *thurberiae*: Head and beak: A, Female; B, male. Much enlarged. (Original.)

The egg puncture is almost always made at the base of the square, and the hole is sealed by a gelatinous scale exuded by the plant, over which there is often a small mass of excrement. On removal of this scale the egg can often be seen. A majority of the eggs seen were twice as long as broad, and only one was of the same proportions as usually found in *Anthonomus grandis*. In the bolls the position of the egg puncture is more general.

**DEVELOPMENT.**—The developmental period of the Arizona weevil on its native host has not been studied, but it has been watched by Mr. Coad at Victoria, Tex., on cotton. The period is practically the same as for Texas weevils, beginning on the same day: In June, 16 days; in July, 12.5 days; in September, 17.2 days. The period in bolls in September is naturally longer, and no specimens had been carried completely through at the time of writing this article.

The most interesting point in the Victoria work lies in the fact that in June, when this boll weevil was removed from hibernation and transplanted on cotton, it was able to begin its generations immediately and to continue reproduction throughout the season.

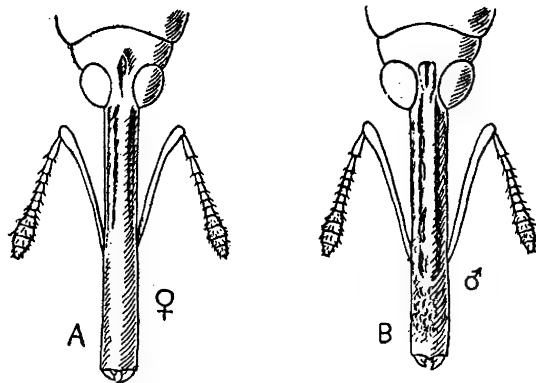


FIG. 4.—*Anthonomus grandis* Boh.: Head and beak: A, Female; B, male. Much enlarged. (Original.)

The food plant of this new variety is known botanically as *Thurberia thespesioides*, although it has also been called *Gossypium thurberi* and *Ingenhouzia triloba*. It occurs in southwestern Chihuahua and Guadalupe, Mexico; in the Santa Catalina, Santa Rita, Tanque Verde, Rincon,



Mule Pass, Huachuca, and Chiricahua Mountains, and also in Fish Creek Canyon of the upper Salt River valley, and at Dagoon, Fort Bowie, and Davidson Springs, all in Arizona.

Thurberia grows at altitudes from 2,250 feet to 7,000 feet, and is found in the bottom of the canyons, on the canyon walls, and on top of the ridges, growing usually where protected more or less from the greatest heat of the sun.

The plant begins flowering in some localities in July, but in others it is just beginning to bud in the latter part of August. Flowering continues into October.

In appearance Thurberia is so nearly like cotton that the Mexicans and natives call it "wild cotton." The leaves are simple, or 3 or 5 lobed, and in the two latter forms resemble the okra-like form of Upland cotton (*Gossypium hirsutum*) or the normal leaves of the Mexican species *Gossypium palmeri* Watt. and *G. schottii* Watt. The leaf has a nectary on the

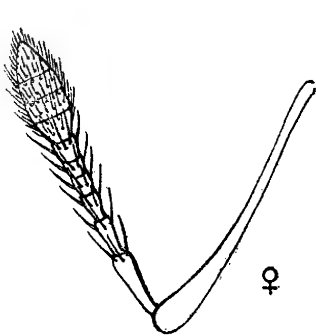


FIG. 5.—*Anthonomus grandis*, var. *thurberiae*: Antenna of female. Much enlarged. (Original.)

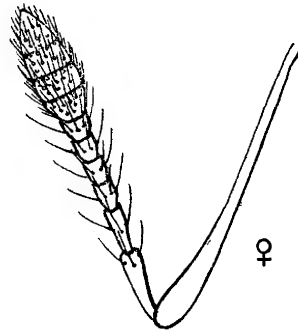


FIG. 6.—*Anthonomus grandis* Boh.; Antenna of female. Much enlarged. (Original.)

midrib, like cotton, and this nectary is as attractive to insect life as the leaf nectaries of Egyptian or Upland cotton. The buds differ from cotton buds by the truncate calyx cup and the linear involucre bracts, but the three nectaries, which also prove a great attraction to insects, are present as on cotton squares. The flowers resemble cotton flowers very closely. The bolls are small, not over three-fourths of an inch in length, and are 3 to 5 celled, with two rows of seed in each. There is a very tiny fiber on the cell walls.

The plants are perennial, growing to be over 10 feet high, with a spread of about 10 feet, and having a large, strong, woody trunk. They are very prolific fruiters. The species is often killed back by frosts, as is evidenced by the dead terminals with the old bolls of previous seasons. The heavy wash in the mountain canyons is one of the principal means of dispersion of the plant.

Thurberia is exceedingly like cotton in most essentials, the relationship being most clearly demonstrated by the many insects which attack both.

At least two species of parasites attack the Arizona "wild-cotton" boll weevil in the Santa Rita Mountains. One of these is a species of *Cerambycobius* and the other is a braconid. There are also some predators which attack it.

Without further information it is idle to speculate as to the direction of the adaptation which has evidently taken place in *Anthonomus grandis*. If further research should locate this boll weevil breeding upon another genus of plants closely related to cotton, such as *Eremoxylum*, a genus of western Mexico, or upon one of the small wild species of *Gossypium* in Mexico, the direction of adaptation might be traced. Some of the

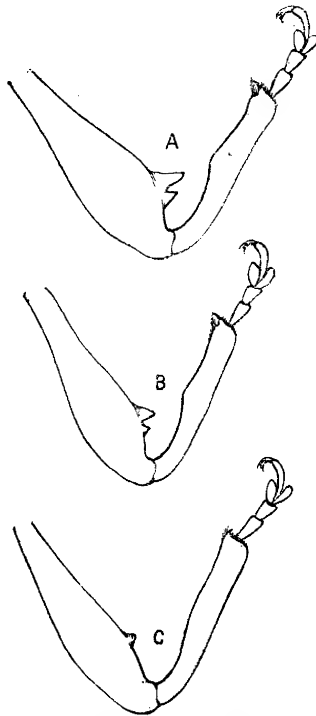


FIG. 7.—*Anthonomus grandis*, var. *thurberiae*: A, Front leg; B, middle leg; C, hind leg. Much enlarged. (Original.)

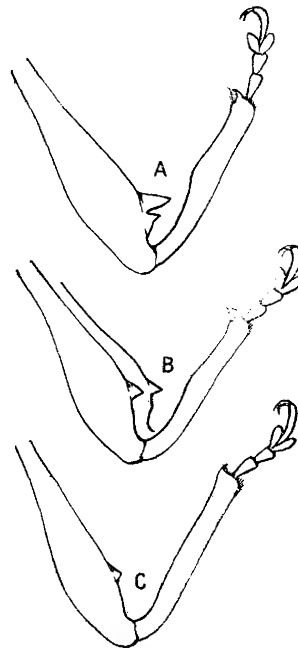


FIG. 8.—*Anthonomus grandis* Boh.: A, Front leg; B, middle leg; C, hind leg. Much enlarged. (Original.)

differences in the condition of the two varieties which show the range of adaptivity of the insect are as follows:

The rainfall in the vicinity of Tucson, Ariz., for 40 years has averaged only 11.66 inches per annum, not reaching 3 inches in any month. July and August are the months of greatest precipitation.

The rainfall at Victoria, Tex., for 20 years has averaged 36.63 inches per annum, with over 3 inches in seven months of the year. May is the month of greatest precipitation.

The rainfall at Opelousas, La., for 17 years has averaged 57.12 inches per annum, with over 5 inches in six months of the year. July is the month of greatest precipitation.

The altitude of Opelousas is 83 feet, of Victoria 145 feet, and of Tucson 2,390 feet. The Arizona boll weevil is found at 4,000 feet altitude and higher. The highest altitude at which the Texas form has been found on cotton is under 2,000 feet.

The maximum temperature at Opelousas and Victoria is  $104^{\circ}$  F., and at Tucson  $112^{\circ}$ . The minimum temperature at Opelousas is  $2^{\circ}$ , at Victoria  $6^{\circ}$ , at Tucson  $10^{\circ}$ . The mean temperature at Opelousas is  $67.3^{\circ}$ , at Victoria  $70^{\circ}$ , at Tucson  $68^{\circ}$ . The average date of first killing frost in the fall for Opelousas is November 17; Victoria, December 10; and for Tucson, November 22. The average date of last killing frost in spring for Opelousas is March 5; for Victoria, February 20; and for Tucson, March 26. At Tucson, August is the only month in which the minimum temperature does not run below  $56^{\circ}$  F., which is the zero of effective temperature for *Anthonomus grandis* in Texas. At Victoria and Opelousas the minimum never goes below  $56^{\circ}$  in July or August.

Of course, in the mountains where *Anthonomus grandis thurberiae* occurs the temperature does not reach quite as high a point as at Tucson, and the minimum temperature is lower. The chilly nights and warm days probably would retard the development and hibernation of the cotton boll weevil in the same manner if transplanted to Arizona mountain conditions.

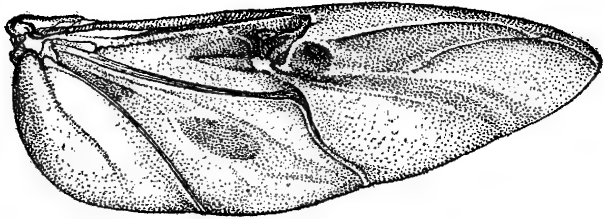


FIG. 9.—*Anthonomus grandis*, var. *thurberiae*: Wing.

The points of greatest adaptation are evidently atmospheric pressure and humidity, and possibly high temperature, although typical individuals of *Anthonomus grandis* have been known to survive  $114^{\circ}$  F. at Dallas, Tex., while the excessive drought experienced for several years in northern Texas practically exterminated the species.

Cotton is cultivated in the Imperial Valley and the Colorado River valley in California, in the Salt River valley, the Gila River valley in eastern and central Arizona, and also in the Santa Cruz River valley of Arizona.

The varieties grown are mainly long staple—Egyptian and Durango, with some Triumph. The crops, which are irrigated, are very promising and can be made with very little water if it is properly applied.

The Arizona "wild cotton," *Thurberia*, occurs in nearly every mountain range in southwestern Arizona where there is any moisture. In the vicinity of the Santa Cruz Valley cotton is grown within 5 miles of *Thurberia* plants growing in the mountains. The boll weevil was not found on the nearest *Thurberia* plants, nor were many of the nearest canyons investigated, but it was found to be abundant not more than 10 miles distant. This is the first year of cotton in the Santa Cruz Valley, and it is expected that a large acreage will be planted in 1914.

Thurberia is known to occur in Fish Creek Canyon, one of the sources of the Salt River. This valley has the most extensive cotton plantings in Arizona. However, the boll weevil has not been observed there.

No observations have been made in the vicinity of the Gila River valley, but as *Thurberia* occurs in the mountains both north and south of this valley, it undoubtedly also occurs in some of the ranges bordering the valley.

The Arizona weevil may be able to cover considerable distances by flight, especially if compelled to seek sustenance elsewhere. However, it will probably cleave to its native food plant as long as this gives sufficiently abundant food, though a great increase of weevils or a decrease of food might drive them to seek other food. They would take more readily to cotton than anything else, and once they find the rich, succulent cotton, with its plentiful food and moistened soil, they will probably do serious damage. It is to be feared that a wholesale destruction of the native food plant might invite a quicker than natural adaptation to cotton on the part of this western weevil. This matter is now under investigation, but at the present time it is the writer's personal opinion that the safest plan is to preserve the status quo of the weevil in the mountains. An introduction of parasites from the cotton boll weevil would be of considerable assistance in reducing the Arizona weevil and would not cause its dispersal.

There is danger of a distribution of weevil-infested buds through the drainage system by summer freshets. After such occurrences the cotton should be watched very closely for several weeks for the appearance of weevils.

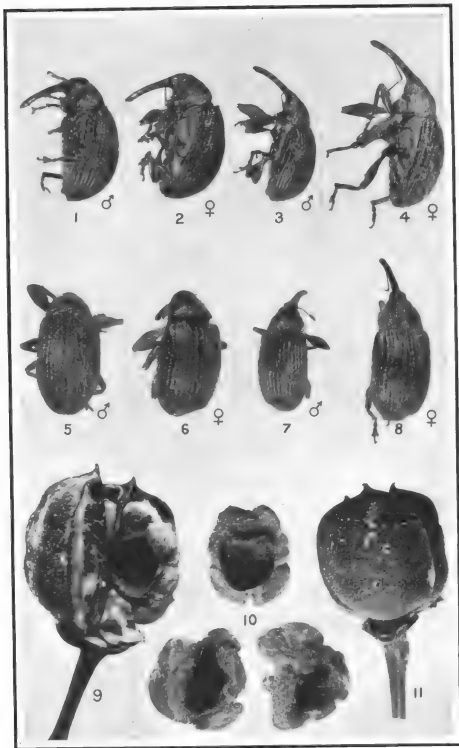
The cotton boll weevil has never been able to successfully invade the drier cotton sections of western and northwestern Texas, although it has been expected that it will gradually adapt itself to the more rigid conditions of these sections. It is of extreme importance that the Arizona weevil be kept out of western Texas and any part of the southeast, except when under very careful isolated observation of specialists. If accidentally introduced into other sections, the *Thurberia* weevil might be able to stand much greater variations of climate than *Anthonomus grandis* Boh. and become a much more powerful pest. Furthermore, there is every reason to believe that *Anthonomus grandis thurberiae* could withstand the rigors of the climate of western Texas.

It is therefore important that restriction by quarantine be considered, and this matter will be taken up at an early date by the Federal Horticultural Board.



#### DESCRIPTION OF PLATE

- PLATE VI. Figs. 1, 2, 5, and 6.—*Anthonomus grandis thurberiae*: Type specimens; actual length, 5.5 to 6 mm. Figs. 1 and 5.—Side and dorsal views of male. Figs. 2 and 6.—Side and dorsal views of female. Enlarged. (Original.)
- Figs. 3, 4, 7, and 8.—*Anthonomus grandis*: Typical specimens; actual length, 5.5 to 6 mm. Figs. 3 and 7.—Side and dorsal views of female. Enlarged. (Original.)
- Fig. 9.—*Thurberia thespesioides*: Section of boll, showing cell of *Anthonomus grandis thurberiae*. Enlarged. (Original.)
- Fig. 10.—*Thurberia thespesioides*: Seed, showing cell of *Anthonomus grandis thurberiae*. Enlarged. (Original.)
- Fig. 11.—*Thurberia thespesioides*: Boll, showing egg puncture of *Anthonomus grandis thurberiae*. Enlarged. (Original.)



# THE DIAGNOSIS OF DOURINE BY COMPLEMENT FIXATION

BY JOHN R. MOHLER, ADOLPH EICHHORN, AND JOHN M. BUCK,  
*Pathological Division, Bureau of Animal Industry*

## INTRODUCTION

Dourine is a specific infectious disease affecting under natural conditions only the horse and the ass, transmitted from animal to animal by the act of copulation, and due to a single-celled animal parasite or protozoan, the *Trypanosoma equiperdum*. It is characterized by an irregular incubation period, the confinement of the first symptoms to the genital tract, the chronic course which it runs, and by finally producing complete paralysis of the posterior extremities, with a fatal termination, as a rule, in from six months to two years.

## HISTORY OF DOURINE IN THE UNITED STATES

In the United States the disease was first suspected in 1885 and recognized in 1886 by Dr. W. L. Williams, who was then a veterinary practitioner at Bloomington, Ill. Officials of the State of Illinois took hold of the outbreak, and as a result of rigid prophylactic measures the disease was eradicated from the State in 1888, but not before an affected stallion had been shipped to Gordon, Nebr., thereby starting up a new center of infection in that locality.

In 1892 dourine was again brought into public notice by an outbreak among the breeding horses of northwestern Nebraska, the history of which suggested that it originated with this Gordon stallion. After an expenditure of about \$5,500 by the Bureau of Animal Industry the disease was considered to have been eradicated from that section of the country. Five years later the infection again made its appearance in the same part of Nebraska, and early in 1899 the Bureau again began the work of eradication. Many inspections were made, and those animals which were found diseased were purchased and killed. Many obstacles were encountered, and the disease evidently kept smoldering during 1900.

In 1901 the infection reappeared with increased vigor, this time in the Pine Ridge and Rosebud Indian Reservations in South Dakota, in addition to northern Nebraska, and more stringent measures were immediately inaugurated to control the spread of the disease. However, eradication in this region was extremely difficult, owing to the wildness



of the country as well as of the horses and the fact that many horse owners would try to conceal from the inspectors animals which they knew to be affected with the disease. In 1906 the last suspicious cases of dourine were destroyed in South Dakota.

In the meantime, during the year 1903, dourine was reported in Van Buren County, Iowa, and successful steps were immediately taken to stamp it out. No connection could be established between this outbreak and that in Nebraska, but it was quite definitely determined that an imported Percheron stallion purchased by a company of farmers was responsible for its appearance.

Another outbreak of dourine was discovered in Taylor County, Iowa, in 1911. The diseased animals, together with all exposed stallions and mares, were immediately quarantined by the State. Those showing lesions of the disease and those exposed horses that reacted to the complement-fixation test were purchased by the Government and destroyed. It is now believed that the infection is entirely eradicated from Iowa. The source from which this center of infection was derived is only a matter of conjecture, but there is apparently no connection between this and any of the previous outbreaks. No authentic information as to the origin of the outbreak was discovered, but all cases lead back to a Percheron stallion which was imported in 1909 and brought direct to Lenox, Iowa.

Early in July, 1912, the State Veterinarian of Montana reported several suspicious cases of dourine in eastern Montana and forwarded blood sera from the suspected animals for the complement-fixation test. All but one sample gave positive results, thus establishing a new center of infection of dourine. From present indications this outbreak appears to be more extensive than any of the previous outbreaks, involving also two Indian reservations in North Dakota and South Dakota; but a force of 12 Federal veterinarians assisted by State representatives is at work on the disease, and the infection is well under control.

#### SEARCH FOR A METHOD OF DIAGNOSIS

The difficulty of diagnosing chronic and latent forms of dourine is generally recognized, and owing to this fact the control and eradication of this disease in horses has been of slow progress and sometimes ineffective. In such outbreaks it has been the custom to trace the disease as far as possible to its origin and then to keep under observation all mares and stallions which directly or indirectly have been exposed to the disease. At the same time animals which show clinical evidences of the affection are destroyed without delay. By this means several of the outbreaks which have occurred in the United States have been checked and eradicated.

The attempt to make a microscopical demonstration of the *Trypanosoma equiperdum* in affected horses is very frequently unsuccessful,

although our more recent experience proves that the organism may occasionally be found in the serous exudate of the plaques and also in the fluid of the edematous swellings of the genital organs in the stallions as well as in the mares.

Of course, this procedure of diagnosis can be attempted only when the disease occurs in farming localities where the animals can be readily observed and examined as desired. On the other hand, in the present outbreak in Montana and adjoining States the conditions make the diagnosis by the demonstration of trypanosomes impossible, and, likewise, animal inoculations can not be satisfactorily utilized for this purpose. Horses in that locality are bred under range conditions; they run wild and a round-up takes place only once a year. The difficulty of an examination, even clinically, of such animals is obvious, since they have not been broken to the halter and are troublesome to handle.

Our experience with the disease in Montana showed that only a limited number of animals were clinically affected. Nevertheless, the association of all the animals without any restriction in the breeding periods indicated that a larger number of animals would be found infected, which, as a matter of fact, has been proved by subsequent tests, as hereinafter shown.

Owing to the fact that until the last few years the eradication of dourine in this country was supposed to have been complete, the disease has received only slight attention as compared with other menacing diseases of our domesticated animals. It was not until the outbreak in the State of Iowa in 1911 that the necessity for devising a method of diagnosing this infection began to be fully realized. The value of being able to detect the latent and to verify the clinical cases became apparent. Otherwise, the necessity existed of maintaining a long-continued quarantine in those sections of the country where cases have been discovered. While little difficulty has been experienced in recognizing the advanced cases, a clinical examination alone naturally permitted many infected animals to escape detection, only to facilitate the further spread of the disease until the appearance of symptoms made the diagnosis unquestionable.

Inasmuch as the complement-fixation method of diagnosis has been employed with gratifying results in connection with numerous other diseases, the possibility of applying this method to dourine naturally suggested itself, and steps were therefore taken to determine the feasibility of its application to this disease.

It was very early discovered that the problem of preparing a satisfactory antigen would offer considerable difficulty. Efforts were primarily directed toward utilizing for this purpose the different organs of those horses that had succumbed to the disease. Several of the clinical cases were shipped from Iowa to the Bethesda Experiment Station during the outbreak referred to, in order that a more complete observa-

tion might be made of the development of the disease and that material might at the same time be available for the preparation of an antigen. From time to time, as these animals died, certain tissues were obtained which it was suspected might furnish the desired results, but although shake extracts of the spleens, livers, kidneys, and bone marrow, as well as alcoholic and acetone preparations, were employed under various conditions, the results were rather discouraging.

Subsequent to this time there came under our observation publications by numerous investigators who had given this subject consideration. It will suffice to mention the publications of Landsteiner, Müller and Pötzl, Levaditi and Yamanouchi, Hartoch and Yakimoff, Citron, Weber, Manteufel, Manteufel and Woitke, Zwick and Fischer, and Schilling, Claus, and Hösslin. The results in these instances appeared to have been unsatisfactory, which was also the case in the extensive work on the diagnosis of dourine by the Wassermann method by Trajan Pavlosévici, as he concluded that while antibodies can be demonstrated by this method in laboratory animals infected with trypanosomes, the method can not be utilized in stallions affected with dourine.

Later, Winkler and Wyschelessky, Mohler, and also Watson in their work on complement fixation as an aid in the recognition of trypanosomiasis indicated the good results obtained in the diagnosis of dourine. Likewise, Mattes in his work on the agglutination of trypanosomes obtained gratifying results, while Braun also concludes that complement fixation can be utilized for the diagnosis of trypanosome affections.

In the recorded publications it was observed that the more promising results were obtained by those who employed suspensions of pure trypanosomes. The organ extracts and other preparations of antigens generally used for this purpose proved unreliable. The procedure as recommended by various workers in obtaining an antigen from pure trypanosomes and using such a suspension as the antigen has also been tried by the writers with uniformly good results. The practical application of this procedure, however, would be very laborious and require a great deal of time, especially in cases where a large number of horses have to be tested by this method. Accordingly it was deemed advisable to devise a means by which an antigen could be prepared which would give similarly good results but would not require such delicate and laborious technique. In place of the specific trypanosome of dourine being utilized, the writers selected the surra organism, as it had been previously ascertained by several investigators that the reaction obtained was not absolutely specific for any one trypanosome infection but was rather of a group nature. As dourine is the only known trypanosome affection of horses existing in this country, the value of even a group reaction was immediately appreciated, and attention was directed to the carrying out of this idea in our diagnostic work.

In place of preparing suspensions of the trypanosomes, however, an antigen was made of the blood and macerated spleens of rats killed at the height of surra infection. This material was placed in a bottle containing glass beads and shaken for six hours, filtered through gauze, and carbolized. The results from this antigen proved satisfactory, and it was used repeatedly on the blood of the horses affected with dourine that were left of the Iowa shipment.

The smallest quantity of the serum which gave a positive reaction with the antigen was 0.05 c. c.; however, the various comparative tests indicated that fixation in tubes containing 0.2 c. c. of serum is sufficient for diagnostic purposes. Sera from normal animals, also those affected with various other diseases, failed to give a reaction. This antigen proved active on 10 consecutive days, but failed to produce fixation of complement on subsequent tests. Later attempts by the same procedure also resulted less satisfactorily, and it was therefore deemed advisable to try other methods in order to procure an antigen of more uniform action.

The following procedure was next employed:

After successive examinations of the blood of a dog infected with surra, about 200 c. c. of blood were drawn from the jugular vein when the microscopic examination revealed an extreme infestation with the parasite. The blood was drawn into a 1 per cent potassium-citrate solution in large centrifuge tubes of 100 c. c. capacity. A quantity of potassium-citrate solution was used equal to the amount of blood drawn into each tube, and 0.5 gram of saponin was added to each tube in order to dissolve the red blood corpuscles. After a thorough shaking and after complete hemolysis had taken place, the tube was centrifuged for 30 minutes at 2,500 revolutions, and the supernatant fluid was siphoned off. The residue, which was of an opaque color and consisted principally of trypanosomes, was then thoroughly mixed and shaken up with salt solution, when it was again placed in the centrifuge; this washing was repeated three times. After the last washing the thrown-down opaque mass was emulsified with 50 c. c. of salt solution and titered as to its merits as an antigen for dourine tests. The results were highly satisfactory, and the titer was established at 0.5 c. c. of this emulsion per tube. However, the disadvantages of this method—namely, the difficulty in the preparation of this antigen and also the small quantity which was obtainable from a single bleeding of a dog—were soon apparent.

In July, 1912, the outbreak of dourine in Montana was discovered, as already mentioned. Several samples of blood sera from clinical cases were forwarded by the State authorities to the Bureau of Animal Industry for verification. Positive reactions were obtained in numerous instances with antigens thus prepared, establishing conclusively the presence of the disease in that State, as well as suggesting the possibilities of the test as a means of its eradication. It was not long before dis-

covery was made that the disease was quite widely spread in Montana owing to the previous failure to recognize it. In an endeavor to comply with the request of the State authorities to make diagnoses in a large number of animals, it was soon apparent that a different method would necessarily have to be devised in order to make the desired progress.

#### PREPARATION OF ANTIGEN

Various organs from rats just dead from surra were tried out in both fresh and preserved states, and the results which were obtained from the fresh suspension of the macerated spleen of a rat just dead from surra were the most promising. In order to establish whether such an antigen would constantly, or at least in most instances, give the results desired, it was repeatedly tested on positive sera of horses affected with dourine, as well as on horse serum known to be free from immune bodies of dourine. After repeated tests on horses clinically affected with dourine had shown the antigen to be uniformly constant in its action, the procedure of diagnosing dourine by this method was definitely adopted. It was at this time that our present method of preparing antigen was first employed, which is as follows:

Gray or white rats are infected with surra by the injection of 0.2 c. c. of blood from a rabbit infected with that disease. Since tests have to be made every day to keep up with the large number of cases submitted and as the antigen proves effective only when prepared fresh, it was arranged that at least two rats should die daily with the disease. When the rats appeared to be at the point of death late in the afternoon it was found that placing such rats in the ice chest until they died furnished a better antigen than when they have died in the cage during the night and have to be used the following morning.

The spleens of the rats are removed, placed in a mortar, and ground up with a small amount of salt solution to a pulpy mass. From time to time more of the salt solution is added, and the suspension thus obtained is filtered twice through a double layer of gauze into a test tube. The quantity of the suspension from each spleen is made up to 40 c. c. by dilution with salt solution.

This suspension constitutes the antigen for the tests of the suspected dourine sera. Dr. Jacob Traum, who was temporarily assigned to this work, found that when the suspension was titered against sera in graduated quantities from a known positive and a known negative case the best results were obtained, and this method has since been adopted. The quantity of antigen employed is double the amount necessary to produce complete fixation with positive serum. The following table gives the method practiced in titrating the antigen:

Table showing method of titration of antigen for the complement-fixation test in dourine.

Positive serum.								
Tube No.	NaCl solution. <sup>1</sup>	Serum.	Antigen. <sup>2</sup>	Comple- ment. <sup>3</sup>	For 1 hour in incubator.	Hemo- lytic serum. <sup>4</sup>	Blood cor- puscles. <sup>5</sup>	For 1 hour in incubator.
	C. c.	C. c.	C. c.	C. c.		C. c.	C. c.	
1	2	0.15	0.05	I	For 1 hour in incubator.	I	I	For 1 hour in incubator.
2	2	.15	.1	I		I	I	
3	2	.15	.15	I		I	I	
4	2	.15	.2	I		I	I	
5	2	.15	.25	I		I	I	
6	2	.15	.3	I		I	I	
Negative serum.								
1	2	0.15	0.1	I	For 1 hour in incubator.	I	I	For 1 hour in incubator.
2	2	.15	.2	I		I	I	
3	2	.15	.3	I		I	I	
4	2	.15	.4	I		I	I	
5	2	.15	.5	I		I	I	
6	2	.15	.6	I		I	I	

<sup>1</sup> 0.85 per cent NaCl solution.<sup>2</sup> Suspension of macerated spleen from rat.<sup>3</sup> The determined smallest quantity established by titration.<sup>4</sup> Sensitized rabbit serum.<sup>5</sup> 5 per cent suspension of red blood corpuscles of sheep.

Half the quantity of antigen which in the negative serum does not inhibit hemolysis, provided this quantity is at least double the amount necessary to produce complete fixation with the positive serum, indicates the titer of the antigen. For instance, if tubes Nos. 1, 2, 3, and 4 of negative serum show complete hemolysis and Nos. 5 and 6 slight inhibition, and at the same time tubes Nos. 6, 5, 4, 3, and 2 of positive serum show complete fixation and No. 1 partial fixation, the quantity of antigen for the test proper would be 0.2 c. c. of the antigen.

Occasionally the antigen does not prove satisfactory for the test and has to be discarded. In these cases the fixation in all tubes is apparently due to the excessive amount of proteids from the spleen. Experience has shown that the excessively large spleens contribute such an antigen. This, of course, is indicated by the titration undertaken prior to the regular test. At other times it was found that the antigen proved satisfactory the following day, after it was allowed to stand in the test tube overnight and the supernatant fluid drawn off for the antigen. This is then retitered and the titer established in accordance with the results of the test.

#### THE COMPLEMENT-FIXATION TEST

The test proper for the diagnosis of dourine is carried out in a manner similar to that practiced for the diagnosis of glanders.<sup>1</sup>

<sup>1</sup> A more detailed description of the technique of this method as applied to glanders is given by Mohler and Eichhorn in Bulletin 136, Bureau of Animal Industry, entitled "The diagnosis of glanders by complement fixation."

The hemolytic system consists of sensitized rabbit serum, serum from a guinea pig, and a 5 per cent suspension of washed sheep corpuscles.

The serum to be tested is, of course, inactivated for one-half hour at 56° C. and is used in the tests in quantities of 0.15 c. c., since it has been found that fixation in this quantity is obtained only with sera of horses affected with dourine. Tests to determine the smallest quantity of serum of horses having dourine which will give a fixation showed that in several instances even 0.02 c. c. of serum was sufficient to give a complete fixation.

The complement from the guinea pig is always titered previous to the test, as it is absolutely necessary to use the exact amount of the complement to obtain the best results, since a deficiency or an excess of the complement would interfere greatly with the reaction.

In the numerous cases which have been tested the results were almost invariably definite, and only on a very few occasions was it found necessary to make retests on cases which appeared atypical. The reaction is always very marked, and in our work only a complement fixation with the quantity of serum mentioned is recognized as a positive reaction. It is only proper that in the tests the usual number of checks should be employed in order to insure reliable results.

Since the testing has been undertaken by the method described, 8,657 samples have been examined from Montana and the Cheyenne and Standing Rock Indian Reservations in North Dakota and South Dakota. Of these, 1,076 gave positive reactions, which appears to be a very large proportion, but when it is remembered that these animals were kept under range conditions without sanitary or veterinary control and also that before the disease was recognized as dourine it had been diagnosed for a long period as some other affection, it will be apparent that the opportunity for the spread of the disease was ideal.

With the present system of diagnosis, by which even the latent cases can be determined, it is hoped to eradicate the disease quickly. All the horses in the infected localities will be submitted to the complement-fixation test, and by cooperation with the State authorities means will be devised to dispose of the affected animals in such a way as to make the further spread of the disease impossible. The animals which were destroyed as a result of the disease in the above-named localities and which were diagnosed by the complement-fixation test showed in most instances some lesions indicative of the disease. In some of the cases there were no indications of a progressive paralysis, but the lesions existing in the genital organs of either the male or female were sufficient for confirmation of the diagnosis by the complement-fixation test.

It is therefore evident that the diagnosis of trypanosome infections of both man and animal by the complement-fixation test is of very great importance, especially in countries where only one of these protozoan

diseases exists. By this means it is possible to determine all infected persons or animals within a short time and adopt such hygienic measures as would be best suited for the control of the infection. Furthermore, the introduction of a disease like dourine into any country could also be guarded against by a compulsory requirement of this test on all horses imported from countries in which dourine is present.

## BIBLIOGRAPHY

- BRAUN, H. Über das Verhalten der Trypanosomen Antikörpern gegenüber. *Centbl. Bakt. [etc.]*, Abt. 1, Ref., Bd. 54, Beil., p. 11-16, 1912.
- CITRON, JULIUS. Die Komplementbindungsversuche bei Erkrankungen mit bekannten, aber nicht züchtbaren Erregern. Kraus, Rudolf, and Levaditi, C.: *Handbuch der Technik und Methodik der Immunitätsforschung*, Bd. 2, Jena, 1909, p. 1112.
- HARTOCH, O., AND YAKIMOFF, W. Zur Frage der Komplementbindung bei experimentellen Trypanosomen. *Wiener Klin. Wchnschr.*, Jahrg. 21, No. 21, p. 753-755, Mai 21, 1908.
- LANDSTEINER, K., MÜLLER, R., AND PÖTZL, O. Über Komplementbindungsreaktionen mit dem Serum von Dourinetieren. *Wiener Klin. Wchnschr.*, Jahrg. 20, No. 46, p. 1421-1422, Nov. 14, 1907.
- Zur Frage der Komplementbindungsreaktionen bei Syphilis. *Wiener Klin. Wchnschr.*, Jahrg. 20, No. 50, p. 1565-1567, Dez. 12, 1907.
- LEVADITI, C., AND YAMANOUCHI, T. La réaction des lipoides dans les Trypanosomiasés et les spirilloles experimentales. *Bul. Soc. Path. Exot. [Paris]*, t. 1, No. 3, p. 140-144, 1908.
- MANTEUFEL. Untersuchungen über spezifische Agglomeration und Komplementbindung bei Trypanosomen und Spirochaeten. *Arb. K. Gsndhtsamt. [Germany]*, Bd. 28, Heft 1, p. 172-197, März, 1908.
- AND WORTHE. Über die diagnostische Bedeutung der Komplementbindungsreaktion bei Trypanosomeninfektionen. *Arb. K. Gsndhtsamt. [Germany]*, Bd. 29, Heft 2, p. 452-477, 1908.
- MATTES, WILHELM. Agglutinationserscheinungen bei den Trypanosomen der Schlafkrankheit, Nagana, Dourine, Beschälseuche, und des Kongoküstenfiebers. *Centbl. Bakt. [etc.]*, Abt. 1, Orig. Bd. 65, Heft 6/7, p. 538-573, Aug. 10, 1912.
- MOHLER, JOHN R. Dourine. Report of committee on diseases. *Proc. Amer. Vet. Med. Assoc.*, 1912, p. 99-115, 1913.
- PAVLOSÉVICI. Recherches sur l'application de la méthode Wassermann, dans le diagnostic de la dourine. *Arch. Vet. [Bucharest]*, v. 7, No. 2, p. 69-82. Mar.-Apr. 1910.
- SCHILLING, CLAUS, AND HÖSSLIN, V. Trypanosomen-Infektion und Komplementbindung. *Deut. Med. Wchnschr.*, Jahrg. 34, No. 33, p. 1422-1425, Aug. 13, 1908.
- WATSON, E. A. The serum reactions and serum diagnosis of dourine. *Proc. Amer. Vet. Med. Assoc.*, 1912, p. 411-420, 1913.
- WEBER, HANS. Über Immunisirungs- und Behandlungsversuche bei Trypanosomenkrankheiten. *Ztschr. für Expt. Path. u. Ther.*, Bd. 4, Heft 2, p. 576-626, 1907.
- WINKLER AND WYSCHESLESSKY, S. Die Agglutination, Präzipitation, und Komplementbindung als Hilfsmittel zum Nachweis der Trypanosomenkrankheiten im besonderen der Beschälseuche. *Berlin. Tierärztl. Wchnschr.*, Jahrg. 27, No. 51, p. 933-936, Dez. 21, 1911.
- ZWICK AND FISCHER. Untersuchungen über die Beschälseuche. *Arb. K. Gsndhtsamt. [Germany]*, Bd. 36, Heft 1, p. 1-103, 1910.





# THREE UNDESCRIBED HEART-ROTS OF HARDWOOD TREES, ESPECIALLY OF OAK

By W. H. LONG,

*Forest Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry*

## INTRODUCTION

During an investigation made in 1912 of the pathological condition of the oaks in the Ozark National Forest, of Arkansas, and in other sections of the United States the writer found a large percentage of the trees, especially in some regions of Arkansas, attacked by various fungi which rot the heartwood. Twenty different kinds of heart-rots were found. Of this number eight have been previously described and assigned to their causative fungi; two were caused by well-known fungi, but no detailed specific descriptions of the rots have yet been published; one proved to be a true root-rot caused by *Polyporus dryadeus*; three have not yet been connected with their causative organisms; while six have been for the first time definitely associated by the writer with the fungi which produce them. Only three of these last six rots will be discussed in this paper.

## INVESTIGATIONS OF HEART-ROTTING FUNGI

The writer found in the Ozark National Forest ideal conditions for the study of heart-rotting fungi, as thousands of white-oak trees (*Quercus alba* L.) were being worked into 36-inch staves for whisky barrels. Trees over 16 inches in diameter were felled and sawed into about 3-foot lengths; these were immediately split into what are known as bolts. As only perfectly sound timber can be used for whisky staves, all rotten, wormy, water-soaked, and stained pieces were rejected and left on the ground where the tree was cut. It was therefore very easy to determine the character and extent of the rot in each tree. As the areas being cut were in a virgin forest, all ages of trees down to about 160 years old (16 inches in diameter) were included. The majority of the trees were cut very close to the ground; the stumps averaged 12 inches in height, but in many cases were much lower. This aided in the investigation, since the nearer the ground the trees were cut the more complete was the record as to the rot in the trunks.

Of the twenty rots found in oak, only the following eight were present to any extent in the trunks and tops of the trees:

- (1) A rot which produces hollows caused by *Hydnum erinaceus*;
- (2) a brown, checked rot caused by *Polyporus sulphureus*; (3) a

whitish heart-rot, piped in its earliest stages and common in the upper half of the trees, due to *P. dryophilus*; (4) a string and ray rot in the butts of the trees, due to *P. berkeleyi*; (5) a straw-colored rot caused by *P. frondosus*; (6) a white piped or pocketed rot caused by *P. pilotae*; (7) a brown, brittle rot, cause unknown; and (8) a tough, spongy, whitish rot caused by *Fomes lobatus*.

Of these eight rots the bulk of the damage to the timber in the butts of the trees is caused by the following fungi, named in the order of their importance: *Hydnum erinaceus*, *Polyporus pilotae*, *P. sulphureus*, *P. berkeleyi*, and *P. frondosus*. However, *P. dryophilus* causes a most common and very injurious heart-rot of the upper trunk and limbs of oaks in the Ozarks.

Although 64.8 per cent of the felled oak trees studied in the Ozarks were affected with butt-rots, the amount of merchantable timber actually destroyed by these fungi was comparatively small, owing to the fact that these rots do not ascend very high in the trees. More than 2,100 felled oak trees were carefully studied by the writer, and extensive data concerning each tree were recorded. Of the entire number 1,938 were white oaks.

Table I shows the various heights of each rot in the trees down to a certain limit, together with the corresponding stump diameter, the diameter of the rot for each tree, and the number of trees for each recorded rot height. For example, the first line, reading across the page, shows the name of the rot—"hollow-producing rot"; cause—"*Hydnum erinaceus*"; diameter of the stump—"26 inches;" diameter of the rot in the stump—"17 inches"; height of rot in the bole of the tree—"28 feet"; and the number of trees with this height of rot—"1." Where more than one tree has a particular rot of a given height the diameters of the stumps and the diameters of the rot in the stumps are averaged, and the resulting numbers are shown in the proper columns.

TABLE I.—Data on five types of butt-rots found in white oak (*Quercus alba* L.).

Name of rot.	Cause.	Diameter of stump.	Diameter of rot in stump.	Maxi- mum height of rot in butt.	Number of trees having rot of the given height.
		<i>Inches.</i>	<i>Inches.</i>	<i>Feet.</i>	
Hollow-producing rot...	<i>Hydnum erinaceus</i> ...	26	17	28	1
		40	36	24	1
		30	27	20	3
		25	21	19	1
		29	26	18	2
		26	19	17	1
		29	23	16	2
		32	27	15	3
		26	20	14	4
		28	21	13	3
		28	21	12	10
		30	22	11	2
		27	20	10	13
		40	36	24	1
		28	26	20	1
Pocketed or piped rot...	<i>Polyporus pilotae</i> .....	29	24	16	1
		30	25	15	3
		29	23	14	1
		28	21	12	5
		26	23	10	3
		29	27	18	1
		33	24	12	2
Brown, checked rot....	<i>Polyporus sulphureus</i> ..	36	29	9	1
		26	19	8	4
		26	22	7	4
		28	24	6	19
		38	32	13	1
String and ray rot.....	<i>Polyporus berkeleyi</i> ...	30	27	10	1
		28	21	8	2
		28	17	6	3
		27	20	5	2
Straw-colored rot.....	<i>Polyporus frondosus</i> ...	29	23	4	2
		25	17	3	3

TABLE I.—Data on five types of butt-rots found in white oak (*Quercus alba* L.)—Contd.

## SUMMARY.

Name of rot.	Cause.	Average—				Total number of trees infected.
		Diameter of stump.	Diameter of rot in stump.	Height of rot in butt.	Age of rot.	
Hollow-producing rot.	<i>Hydnum erinaceus</i>	Inches. 26. 0	Inches. 12. 6	Feet. 3. 9	Years. .....	648
Pocketed or piped rot.	<i>Polyporus pilotae</i> ..	25. 6	13. 7	3. 9	156	408
Brown, checked rot.	<i>Polyporus sulphureus</i> .	25. 8	13. 6	3. 0	.....	270
String and ray rot..	<i>Polyporus berkeleyi</i> .	28. 0	19. 0	3. 5	190	57
Straw-colored rot..	<i>Polyporus frondosus</i> .	27. 0	14. 0	2. 3	.....	12

In the above summary are given certain data for each of the most important butt-rotting fungi in white oaks, and from them some idea can be obtained as to the amount of damage done by these heart-rotting fungi in the virgin timber of the Ozark National Forest. All of the rots listed in the table are also found in black oak (*Quercus velutina* Lam.), as well as in white oak, but on account of the limited number of trees of this species examined no data are now given for it. All height and diameter measurements given in this article, unless otherwise stated, were taken from the tops of stumps 12 inches high.

In determining the age of the rot only trees were used in which the fungus had undoubtedly entered at an old fire scar long since healed over. The annual rings of wood were counted from the point where the callus had completely closed the wound, so that the heart-rotting fungus must have entered before the wound was covered. Therefore, the figures given here represent the minimum age for each infection. The rot might have entered sooner and therefore be older, but it could not have entered later and therefore be younger, as the callus had closed the wound. No stumps with open wounds of any kind were used in estimating the age of the rot.

The writer realizes that this method of determining the length of time the fungus has been in a tree is open to the following criticism:

(1) The fungus might have entered underground through injuries which reached to the heartwood of the root and thence moved upward into the bole of the tree; (2) the wound made by the fire may have healed above ground, but not below on the stool and roots of the tree, thus

leaving a permanent opening into the heartwood of the trunk just below the surface of the ground. Through such hidden openings the mycelium of any heart-rotting fungus capable of growing in the forest débris could enter the tree, and thus the resultant rot would be directly associated with the old fire scar; (3) some of these heart-rotting fungi may be able to enter through sound, unbroken living roots and then move upward as a heart-rot into the bole of the tree. In this case they would also be true root parasites and not simply mere heart-rotting fungi.

None of the three rots discussed in this article are known to be true root parasites. As to the first objection mentioned, the writer has investigated several hundred uprooted oak stumps, many of which had heart-rot, and in no instance was any evidence found indicating that the heart-rotting fungus entered through the roots and thence worked upward in the tree. On the contrary, repeated instances were found where the rot began at the surface of the ground in an old fire scar or other wound and moved downward in the heartwood of the root and upward in the bole of the tree. In every case where the rot had entered the roots it had evidently come from above and not from below, as the rot was limited to the heartwood of the root, while the sapwood was alive and sound. However, there is a large wood borer which lives in the roots of oaks, and when its burrows reach the surface of the roots an opening would be made for any fungi to enter from the soil. It is well known that in the roots of oaks the amount of heartwood compared to that of sapwood is very small. This in itself makes improbable the entrance of heart-rotting fungi through the roots, especially sound ones.

In regard to the second objection mentioned, the writer has recently examined more than 200 oak trees with fire-scarred bases, and not a single one was found in which the wounds having healed above ground had not also completely healed over below the ground. As a rule, forest fires injure the tree but a short distance, 2 to 3 inches, below the collar of the tree, owing to the protection of the soil. Therefore, it is not impossible for these three heart-rotting fungi to enter through the root system; but taking the above facts into consideration it is improbable that they did enter by this route, even granting that they are capable of leading a purely saprophytic existence in the soil and forest débris—a condition yet to be proved.

The very close association of the heart-rots with the old fire scars in the trees studied is so evident that undoubtedly the causal fungi entered the tree by this route. So marked is this association of fire scars with heart-rots in the Ozarks that one could tell the areas in the forest which had been most frequently burned over from the percentage of trees affected with heart-rots.

The writer has found three types of heart-rotting fungi in living trees:

(1) Those limited to the base and lower portion or butt of the tree, for example, *Polyporus berkeleyi* and *P. frondosus*; (2) those which are able

to enter either at the butt or in the top of the tree, such as *Hydnum erina-ceus*, *Polyporus sulphureus*, and *P. pilotae*; (3) those which enter the upper portion of the tree and work in both directions from the point of entrance, but rarely, if at all, enter through fire scars at the butt, such as *P. dryophilus* and *Fomes everhartii*.

### THREE UNDESCRIBED TYPES OF HEART-ROTS

In a later article the writer expects to discuss a large number of heart-rots of the oak, limiting this paper to a detailed description of the following rots: A pocketed or piped rot of the oak, chestnut, and chinquapin caused by *Polyporus pilotae*; a string and ray rot of the oak caused by *P. berkeleyi*; and a straw-colored rot of oak caused by *P. frondosus*.

#### A POCKETED OR PIPED ROT CAUSED BY POLYPORUS PILOTAE

The rot produced by *P. pilotae* has been found by the writer directly associated with the sporophores of this fungus in the following species of trees: *Quercus alba* L., *Q. velutina* Lam., *Q. texana* Buckl., *Q. coccinea* Muenchh., *Castanea pumila* (L.) Mill., and *C. dentata* (Marsh) Borkh.

#### A POCKETED OR PIPED ROT IN WHITE OAK

The description of the pocketed or piped rot which follows was made from the diseased wood of a white-oak tree (*Quercus alba*) which was cut on July 23, and on August 27 the sporophores of *Polyporus pilotae* shown in Plate VII, figure 1, were found fully developed on the end of the log. There could be no question as to the identity of the fungus producing the rot in this case, as less than 30 days had intervened between the felling of the living tree and the formation of the sporophore of *P. pilotae*.

The first indication of this rot in white oak is a slight browning of the heartwood. Later white, oval, or circular cellulose patches from delignification appear in this discolored wood. These white areas by dissolution of the fibers often become holes, which show in both radial and cross section (Pl. VII, fig. 2). The delignification seems to originate in the last layers of the summer-wood fibers and spreads in a very irregular manner. In later stages long strings of white cellulose fibers are found. This is especially true where an abundance of air and rain water can reach the rotting area, especially in old dead logs or in trees with cracks or in hollow, open butts. The delignification and absorption of the fibers do not follow the spring wood as closely as they do in the scarlet oak (*Quercus coccinea*).

Another type of cavity may be formed which seen in radial view is 0.5 to 1 mm. by 1 to 2 mm. in size. These cavities are lined with the ends of the white cellulose fibers and usually occur in and at right angles to the large spring vessels, but they may also extend radially from one annual ring to the next in a more or less winding or interrupted course.

Under the microscope the large, thick-walled, colorless hyphæ are plainly seen in these holes, and to them the holes undoubtedly owe their origin. The edges of the perforated vessels as well as the adjacent cells have been delignified. This type of cavity was especially abundant in the wood immediately adjacent to the sporophore.

The final stage of this rot in white oak seems to present one of two conditions: If an abundance of air and water is present, all the wood fibers will be changed to cellulose, then dissolved, leaving a very light, brittle, rotted wood of a dark-brown color, which later gradually crumbles into a dirtlike mass. This is the type of rot usually found in dead trees or living trees with hollow, open butts. If, on the other hand, only a limited amount of air and no rain water is present, as is the case in living trees with no open wounds reaching to the diseased heartwood, the rotting wood may become honeycombed with empty, cellulose-lined, elliptical cavities (Pl. VII, fig. 3) or it may decompose into a fibrous mass consisting of long, white cellulose strands and partially decomposed vessels and medullary rays. Large quantities of these white cellulose strands are often found in the butts of freshly cut trees which externally appear perfectly sound but have this rot in the heartwood.

#### A POCKETED OR PIPED ROT IN SCARLET OAK

The following description of the pocketed or piped rot was made from a wind-thrown scarlet oak (*Quercus coccinea*), which on falling split on the upper side for 7 or 8 feet. From this fissure a sporophore of *Polyporus pilotae* protruded. The rot began in the top of the tree and had reached the ground. The tree was sawed into 6-foot lengths and split open on March 5, and on May 30 fresh sporophores were beginning to form on the ends of the split pieces.

In this host the fungus first attacks the spring wood immediately around the larger vessels, turning it to a light-tan color. This change in color is accompanied by the absorption, more or less irregularly, of the cells of the spring wood, while the wood fibers intermixed with these cells are delignified from within outward. The tan color of the affected areas is due to the walls of the wood fiber and other cells adjacent to the vessels turning a golden yellow. At this stage of the rot the spring wood is badly decomposed and consists of cells and vessels much eroded, leaving fragments of both intermixed with apparently unchanged cells and vessels. This partial destruction of the spring wood causes it to separate readily into circular sheets along these lines of weakness.

The next stage of the rot going inward toward the center of the tree is the almost complete change of the summer-wood fibers and tracheids into a yellowish white cellulose. Under the microscope the rotten wood is seen to consist of delignified wood fibers intermixed with the remnants of the spring wood and of nearly unchanged medullary rays, while the entire mass of rotted wood is ramified by large, colorless, thick-walled,



much-branched fungus hyphæ 5 to 10 $\mu$  in diameter. These hyphæ are especially abundant in the spring wood. In this stage the rotten wood easily pulls loose in thin flakes, the line of cleavage being between the medullary rays. Many white and yellowish white unabsorbed cellulose wood fibers are found in the rot at this stage.

The third and final stage of the rot is found in the center of the tree and is of a reddish brown color, there being a rather sharp line of demarcation between this and the light-tan color of the second stage. In this last stage there are found remnants of the vessels, a few unabsorbed fiber tracheids, wood fibers, and partially decomposed medullary rays intermixed with the colorless hyphæ of the fungus. Not enough hyphæ are present, however, to bind the rotted wood into a tough mass. The wood at this stage at first is rather brittle when dry and can be partially crushed into fragments between the fingers, but finally it crumbles into a brownish dirtlike mass, which remains in a cavity thus formed inside the tree, unless removed by squirrels, etc. On the split surface of the rotting wood which was exposed directly to the air and rain water a dark, reddish brown mycelial layer of a gelatinous nature was found. This gelatinous mass might, of course, be a foreign growth and not a part of the mycelium of the fungus *Polyporus pilotæ*. The reddish cast is due to the formation of reddish brown bodies on or among the hyphæ; sometimes several of them form a conidialike chain.

In general, the delignification seems to begin in the layer of wood fibers forming the boundary line between the summer growth and the spring layer of wood formed the following year and spreads most rapidly in the spring wood, leaving more or less intact the largest vessels and the cells immediately adjacent. At this stage many of the medullary rays contain a chestnut-brown, humuslike substance.

#### A POCKETED OR PIPED ROT IN THE TEXAN OAK

The rotted wood from which the following description was made was obtained from an old log of Texan oak (*Quercus texana*), just beneath a very large sporophore of *Polyporus pilotæ*.

The rot in this host is much like that described for the scarlet oak, consisting of long strands of white to creamy white, cellulose fibers interspersed with the partially changed spring wood and medullary rays. There is a zone of one-fourth to one-half inch of discolored wood between the sound wood and the zone where the delignification is evident.

#### A POCKETED OR PIPED ROT IN CHINQUAPIN

This description of the pocketed or piped rot was made from material obtained from a fallen log of chinquapin (*Castanea pumila*) on which a sporophore of *Polyporus pilotæ* was found. The rot was seen a number of times in fallen chinquapin trees in the Ozark National Forest. In

living trees of this species, as in the white oak, the rot may vary somewhat.

In the chinquapin the fungus first delignifies the latest formed summer-wood fibers, those immediately adjacent to the large vessels, and spreads finally to all the wood fibers lying between the spring wood of any two successive years. As the summer wood is composed largely of wood fibers, the ultimate result is an almost complete separation of the layers of spring wood. The concentric layers of the spring wood are separated at first by the white to yellowish white, cellulose fibers. Later this cellulose is entirely absorbed, leaving only the concentric layers of the spring wood loosely held together by the remnants of the wood fibers and the few small vessels found in the summer wood (Pl. VII, figs. 4, *a*, and 4, *b*). The vessels and other cells of the spring wood have in the meantime become more or less corroded and have assumed a reddish brown color. In the final stage of the rot the wood when dry is brittle and can be easily broken between the fingers. In old, weathered chinquapin logs attacked by this fungus the rot is very characteristic, consisting of concentric layers of rotten wood which are so loosely held together that one can easily pull off layer after layer.

#### A POCKETED OR PIPED ROT IN CHESTNUT

The material examined for the following description of the pocketed rot was obtained from the diseased wood of living chestnut trees (*Castanea dentata*) located near New Berlin, N. Y. In the hollow butts of these trees the resupinate form of *Polyporus pilotae* was found. Some trees were examined which had recently been made into railroad ties. Ample opportunity was thus given for a thorough study of the various stages of the rot in different regions of the tree trunks.

The first indication of the rot is a watery brownish discoloration of the heartwood. In cross section this discolored area or "soak" often appears as a central circular patch (Pl. VII, fig. 5), often flanked by one or more very narrow crescent-shaped discolored areas, lying between the diseased portion and the sapwood, or sometimes the "soak" may be eccentrically placed in the heartwood of the tree. These rings of diseased wood follow very closely certain annual rings and usually appear first in the immediate vicinity of the large spring vessels. Sometimes only one annual ring will show the disease, and this may extend for several feet longitudinally in the tree beyond that portion of the rot where delignification is evident.

The mycelium of the fungus travels much more rapidly longitudinally in the tree than radially. It is first seen in the large spring vessels. The adjacent wood fibers soon show signs of delignification, which usually occurs most abundantly in the latest formed summer wood, where small, irregular, oval patches of cellulose are produced. These patches usually lie opposite the largest vessels and immediately adjacent to them. This association of cellulose and large vessels is especially noticeable in cross

section, where the delignified areas may usually be seen in the summer wood. The delignification may continue without much absorption of the cellulose till long white bands of cellulose are found lying alongside of the vessels. This formation of bands of cellulose is especially marked when an abundance of air and rain water can penetrate the rotting wood. Such a condition obtains in fallen logs with large hollows or cracks in them.

If, on the other hand, the rot is in the center of the heartwood of a living tree, the small, oval-shaped cellulose patches increase in size, hyphæ from the adjacent vessels gradually absorb the cellulose until lens-shaped cavities are formed which at first are filled by a dense growth of rather coarse hyaline hyphæ. The sides of these cavities are lined with the projecting ends of the delignified wood fibers much like the rot produced by *Trametes pini*. Later both the hyphæ and the cellulose lining may disappear and leave an empty cavity, thus producing a pocketed or honeycomb type of rot.

In the earlier stages of the rot the diseased heartwood surrounding the white cellulose patches is of a cinnamon color. The wood at this stage of the rot is rather firm, contains small cellulose patches (Pl. VII, fig. 6), and has vessels filled with colorless hyphæ from 6 to 10 $\mu$ , or even less, in diameter. The white, cellulose, oval areas gradually encroach upon the summer wood till they extend from one annual layer of vessels to the next. By this time much of the cellulose has been absorbed, and small, distinct cavities are formed. At this stage of the rot the diseased wood is much lighter in weight and can easily be broken into pieces between the fingers. Finally, a condition is reached in which the reddish brown rotten wood is very loosely held together and tends to split up into concentric sheets corresponding to the annual rings. Short oval holes running radially through two or three annual layers of wood are also common at this stage. In rare cases the cells surrounding the vessels are completely absorbed, while the summer-wood fibers are delignified without the formation of cavities. Many of the trees attacked by this fungus had hollows in them, but whether the hollow was caused by this fungus or by a subsequent attack of another fungus, as *Hydnum erinaceus*, could not be determined. While this rot is a butt rot of the chestnut, it is also able to enter through dead limbs and thus produce a top rot. The rot when it enters by means of a dead branch follows the heartwood of the branch down to its juncture with the heartwood of the tree. The fungus then travels both upward and downward in the bole of the tree (Pl. VIII, fig. 1).

Of the chestnut trees in the region examined around New Berlin, N. Y., fully 75 per cent had tops attacked by this fungus. This large percentage was probably due to numerous dead limbs on each tree, thus affording the fungus ample opportunity to enter the tops. Of 302 felled chestnut trees which were studied by the writer in this region 119, or 39.4 per cent, had this rot in the butts. This large percentage of infection

was mainly due to the fact that practically all of the trees came from a coppice growth, and if the original stump was diseased, the later generation of trees springing from its base were also infected through their union with the old diseased stump. Officials of the Unadilla Railroad claim that chestnut ties having only a small amount of this rot in their centers last only three to five years when placed in their roadbed.

This rot in the chestnut is apparently identical with the piped rot of chestnut described by Von Schrenk and Spaulding.<sup>1</sup> Their description of the piped rot of the oak in the same publication apparently includes two distinct rots; viz, this rot caused by *Polyporus pilotae* and the common heart-rot of the oak caused by *Polyporus dryophilus*,<sup>2</sup> which is also a piped rot in one of its stages and will be described in a later publication.

#### RESULTS OF INVESTIGATIONS OF THE POCKETED OR PIPED ROT

The most common and constant characters of this rot, taking all the hosts into consideration, are the presence of long, continuous strands of cellulose, the delignified wood fibers and fiber tracheids, and the white-lined pockets so common in the living oak and chestnut in the early stages of the rot. In the white oak the changing of the wood fibers into cellulose is not so complete as in the other hosts, so that the wood is not broken down as much. In both white oak and chestnut there are holes which run tangential to the tree through the spring wood or radially from one annual ring to another. This condition is especially noticeable in the older stages of the rot in the butt of the trees and in the vicinity of freshly formed sporophores of the fungus.

Sporophores of *Polyporus pilotae* were formed on living white oaks, on the ends of white-oak logs cut only one month, on old logs which evidently had been cut for several years, on a standing fire-killed yellow oak (*Quercus velutina*), on a fallen and very rotten log of Texan oak (*Q. texana*), on the trunk of a wind-thrown scarlet oak (*Q. coccinea*), on old dead logs of chinquapin (*Castanea pumila*), on the inside of a hollow in a living chinquapin tree, and on chestnut trees (*C. dentata*). In the last instance the sporophores were resupinate and growing in the hollow butts of the living trees. Of the 302 chestnut trees studied in New York 119 had this rot. The average diameter of the rot per tree was 6.5 inches, the average diameter of the stump 16.6 inches, and the average height of the rot per tree was 5.4 feet. The maximum diameter and height of the rot in any one tree was found in a tree 27 inches in diameter. The diameter of the rot in this tree was 20 inches and the height of the rot was 20 feet.

<sup>1</sup> Schrenk, Hermann von, and Spaulding, Perley. Diseases of Deciduous Forest Trees. Bur. Plant Indus., U. S. Dept. Agr., Bul. 149, p. 39, 1909.

<sup>2</sup> Hedgcock, George G. Notes on some diseases of trees in our national forests. Phytopathology, v. 2, no. 2, p. 73, 74, Apr., 1912.

A comparison of the average height of this rot in the chestnut (5.4 feet) with its average height in the white oak (3.9 feet) shows that it extends higher up the bole in chestnut than it does in white oak. This difference is still further accentuated by the difference between the average diameter of the diseased chestnut trees (16.6 inches) and that of the diseased white oak (25.6 inches). The average age of the chestnut was probably not over 100 years, while that of the white oak was about 250 years. The very large and numerous vessels in the chestnut made it possible for the fungus to travel to greater heights in this wood in a given time than it could in the white oak, which is a much denser, slower growing wood. Of course, the amount of rainfall and other environmental factors would have to be taken into consideration when comparing the relative heights of this rot in the chestnut and oak.

On the same area in New York where the chestnut mentioned above was studied, a record was made of 477 felled white oaks. Of this number only 4, or less than 1 per cent, had the piped rot so common in the chestnut. Its average height in these 4 trees was 3 feet, its average diameter was 8 inches, and the average diameter of the affected trees was 15 inches. This small percentage of infection was probably due to the fact that no fires had been allowed in these woods and therefore practically no opening into the heartwood of the trees was offered and to the further fact that the oaks did not originate from a coppice growth.

On an area in Virginia which had been in timber for about 60 years the writer checked the stumps of 565 chestnut trees which had been recently cut. The majority of these trees originated from sprouts and had made a vigorous growth, the average age of the trees being about 50 years. Of the 565 chestnut trees only 18, or 3 per cent, had piped rot in the butts. Of this same area 201 white-oak stumps were also checked, of which number 13, or 6 per cent, had piped rot in the butts. This area was an old abandoned field which had been used as a pasture for many years and, so far as the writer could ascertain, had not been burned over in 50 years.

The rate of growth of the various rots in individual trees, as shown by the records made in the Ozarks, varies greatly. For instance, *Polyporus sulphureus* had been in one white oak 200 years and had made a growth in height of only 6 inches during that time, while the same fungus had been in another white oak for 50 years and had made a growth in height during that time of 3 feet. A similar wide range in growth is found for the rot produced by *P. pilotae* in white oak, where it was in one tree for 280 years and had made a growth in height of only 6 inches, while in another white oak the same fungus had made a growth of 4 feet in only 60 years. However, taking into consideration the average and maximum height of each of these rots and their average rate of growth in a tree, it is evident that they do not grow with any thing like the rapidity—at least in white oak—that might be expected.

Of the 1,938 white oaks studied in the Ozarks 408 trees had this rot. The average diameter of the rot per tree was 13.7 inches, the average diameter of the stump was 25.6 inches, and the average height was 3.86 feet. The maximum diameter and height of the rot in these trees was found in a tree 400 years old. The diameter of the tree was 40 inches, the diameter of the rot was 36 inches, and the height of the rot was 24 feet. The oldest rot was 280 years and was found in a tree 310 years old. The average age of the rot in 92 trees was 156 years. The average rate of growth of the rot was 1 foot in height and 3.5 inches in diameter for every 40 years of time. The youngest white oak found with this rot was 180 and the oldest 400 years old.

The exact range of this fungus is not known. It is very common in oak and chinquapin in the Ozark National Forest and has been found in Virginia on scarlet oak.

The writer has also examined authentic sporophores of this fungus on the following hosts and from the following localities:

"On underside of log" (resupinate sporophore), Pennsylvania; "on log," North Carolina; "in hollow oak log," Ohio; "on rotten oak log," Indiana; "on underside of old log" (resupinate sporophore), West Virginia; "on dead oak logs," New York; "on oak," North Carolina; from Iowa, no host given; "on punky chestnut log," no locality given; from Florida, no host given; from South Carolina, no host given; from Tennessee, no host given; "on end of log," Canada; "on oak," Canada; "on old logs," Canada. Three specimens were also seen from Europe, where it is known as *Polyporus croceus* (Pers.) Fries: "On living oak," Sweden; "on old oak and chestnut," apparently from France, no locality given; and "on old oak," locality not given. It probably occurs east of the Rocky Mountains in the United States on oak, chinquapin, and chestnut wherever the hosts grow and also in Europe on oak and chestnut. It is by far the worst heart-rot found in chestnut timber, occurring in this host as both a butt and top rot. It stands second in destructiveness to white-oak timber in the Ozark National Forest, both as to number of trees infected and height attained in the tree. *Hydnum erinaceus* is the most destructive heart-rotting fungus of the oak found in the Ozark forests (see Table I, p. 111). The rot caused by *P. pilotae* was found associated with the rot produced by *Hydnum erinaceus* in 105 trees, with string and ray rot in 3 trees, with *Polyporus sulphureus* rot in 8 trees, and with both *Hydnum erinaceus* and *Polyporus sulphureus* rots in 5 trees.

The sporophores of *P. pilotae* were in an actively growing stage during the month of September in the Ozark National Forest. This fungus usually enters the oak at the base of the tree, probably through fire scars in most instances. The rot was also found occasionally in the upper part of the tree, while the base was not infected. The fungus,

therefore, can enter the tree through fire scars in the butt and also through broken branches or other wounds on the bole and in the top of the tree. There is also a honeycomb rot in oak and in chestnut caused by a species of *Stereum*. This honeycomb rot in its earlier stages resembles so closely certain stages of the rot caused by *P. pilotae* that it is very difficult to determine which fungus produced the rot, unless the sporophores are present.

#### A STRING AND RAY ROT OF OAKS CAUSED BY *POLYPORUS BERKELEYI*

The initial stage of the string and ray rot in the white oak when seen in a radial longitudinal section is characterized by the presence of large amounts of cellulose tissue, causing the rotted wood to have a yellowish white appearance. This stage of the rot may extend for 4 to 8 inches longitudinally, when it terminates rather abruptly in apparently sound wood. The cellulose tissue is composed exclusively of delignified wood fibers, which constitute the bulk of the summer wood. The middle lamellæ have entirely disappeared, so that each delignified wood fiber is separate from its neighbor.

The next stage of the rot is the rather rapid and complete absorption of these delignified fibers, leaving both the spring and summer vessels, the cells immediately adjacent, and the medullary rays intact. The rot at this stage is most characteristic, consisting of a rather dry mass of medullary rays interwoven with long, flat strings of wood (Pl. VIII, fig. 2). These strings are sometimes 8 to 10 inches long by one-sixteenth of an inch wide and consist of the vessels held together by the unabsorbed adjacent cells. The rot in this stage is reddish brown and on account of its peculiar and characteristic structure has been named the "string and ray rot" by the writer. This second stage of the rot may extend from a few inches to several feet up the tree. At first the flattened strings of wood are rather tough, but this gives place to a condition in which the strings get brittle and can be crumbled between the fingers into a brownish, coarse powder. Finally the entire mass of rotting wood becomes overrun with a colorless mycelium. In this condition the rot is very moist, almost wet, and consists of fragments of vessels and of the medullary rays, interwoven with the colorless hyphæ of the fungus. It can now be compressed with the hands into rather firm balls which may be thrown with force and yet will not break into pieces.

Finally the entire mass of rotted wood and mycelium gradually disappears till a hollow is left in the base of the tree. Over the surface of this vanishing mass brittle white or creamy white layers of mycelium are formed, on the undersides of which are cottony masses. Shakes, checks, or worm holes in the wood may have a slight mycelial felt in them.

The string and ray rot seems to be one of the very few heart-rots of the white oak capable of the complete absorption of the heartwood of the tree, thereby producing hollows. The slow rate of travel upward in the

tree compared to its radial rate of growth and the subsequent rather complete absorption of the entire heartwood in the stool of the tree produce a peculiar condition when the tree is cut. A tree in which this rot has reached its last stages in the stool will be rotted to or nearly to the sapwood for 1 to 3 feet from the ground, and such a tree will fall as soon as the thin shell of sound wood is severed, carrying with it the partially rotted heartwood, which easily pulls loose from the badly rotted mass in the stool. The butt end of the felled tree will then have attached to it a cylinder of rotted wood some 1 to 2 feet long in the string and ray stage, thereby leaving a hollow stump in the bottom of which there will be the wet, very rotten mass of wood held together by the threads of mycelium.

This rot has a very strong but pleasant odor, somewhat like that of anise oil. This odor disappears after the exposure of the rot to the air for several weeks, but is so marked when the tree is first cut that it can be detected at a distance of from 20 to 30 feet.

Studies were made of 1,938 white-oak trees which were cut for staves. Of these, 57 had this rot. The average diameter of the rot in these 57 trees was 19 inches; the average height per tree was 3.5 feet; and the average age per tree was 280 years. The maximum diameter and height for this rot in any one tree were found in a tree 380 years old. The diameter of the rot was 32 inches and the height was 13 feet. As a rule, this rot does not extend very high in a tree, as compared to its extent in diameter, and ends very abruptly in perfectly sound wood. It was also found in the butts of two black oaks (*Quercus velutina*); the sporophores of the fungus were seen several times on the roots of both white and black oaks which had not been felled. The writer repeatedly found from one to three sporophores of *Polyporus berkeleyi* (Pl. VIII, fig. 3) attached to the roots of the trees in which this characteristic heart-rot was present. The direct connection of the rot in the stump with the sporophore could easily be traced by following the rot down into the stool and thence through the rotted heartwood of the root to the sporophore. This was done in the case of at least a dozen trees.

The youngest tree found with this rot was 170, the oldest 500 years of age. The rot was usually found in mature and overmature trees from 25 to 32 inches in diameter which grew in rich soil on north slopes. In 6 of the stumps of the 57 white oaks found affected with this rot some evidence as to the age of the rot was obtained. The oldest rot was 380 years and was found in a tree 420 years of age. The average age of the rot in these six trees was 190 years. The average rate of growth of the rot was 1 foot in height and 5.4 inches in diameter for every 60 years of age. The fungus producing this rot usually enters the tree through some wound at the butt, such as fire scars. No evidence was found that it could enter through broken branches. In no instance was the



rot found in the top of a tree. It originates at the butt and travels upward in the heartwood of the tree.

Of the sporophores of *Polyporus berkeleyi* found by the writer all occurred at the base of oak trees, either plainly growing from the exposed root or on the ground near the base of the tree. In the latter case a careful examination of the basal portion of the sporophore showed that it was attached to the roots of the tree. The writer has never found it growing on the bole of the tree above the surface of the ground, though it is not impossible that it could grow as brackets on the trunk, but it is doubtful if it does. *P. sulphureus* Fr. and *P. schweinitzii* Fr., two closely related polypores which produce heart-rots in living trees, are often found growing on the roots at the base of the diseased trees as well as on the boles proper.

There was no evidence to indicate that the fungus could fruit on the trunk after the trees were felled, even if the rot should continue to grow in the felled tree. A small sporophore was found at the base of a 20-foot white-oak snag, while a large sporophore was found at the base of a dead standing white oak, indicating that the fungus could continue to grow and fruit after the trees were dead. The only external evidence that trees are attacked by this heart-rot is the presence of the sporophores of the fungus on the roots. Sometimes the base of the diseased tree is slightly "swell butted." This last character, however, is common to trees attacked by other butt-rots.

This rot was found associated with the rot produced by *Hydnum erinaceus* in 7 trees, with the pocket rot caused by *Polyporus pilotae* in 3 trees, and with the rot produced by *P. sulphureus* in 1 tree. *Hydnum erinaceus* was repeatedly found attacking and completely destroying wood previously rotted by the following fungi: *Polyporus berkeleyi*, *P. pilotae*, *Fomes everhartii*, *Polyporus hispidus*, *P. frondosus*, and *P. dryophilus*, but no evidence was found of its attacking the rot produced by *P. sulphureus*, although it was found associated with this rot in the same tree. Fresh sporophores of *P. berkeleyi* were common during the latter part of August and probably could be found during September. No fresh sporophores were seen in December.

The writer has also examined authentic material of *Polyporus berkeleyi* on the following hosts and from the following localities: "At base of white oak," Canada; "on roots of living white oak," Missouri; from New York, West Virginia, and Missouri no host was given; "from dead place near ground in living oak," Pennsylvania; "on base of stump," North Carolina; "on oak," New York; "on chestnut," New York; "at base of tree," Ohio; "at base of ash stump," Ohio; "at base of oak stump," Pennsylvania; from West Virginia, Pennsylvania, Ohio, North Carolina, and Canada no host was given; "near roots of large oak," Canada; and "under oak," Massachusetts. Apparently this fungus is found only in America. The writer has never seen it growing on anything but oak,

but from the above record it also occurs on chestnut and on ash, while Dr. Weir, of the Office of Investigations in Forest Pathology, reports it on larch in 1913.

From the studies made in the field the writer finds no proof of the ability of this fungus to grow permanently as a saprophyte in humus and decayed forest litter. All sporophores seen certainly grew from mycelium inside the living, diseased trees at whose base they were found and not from mycelium ramifying in and drawing nourishment from the soil or leaf litter.

Weir reports<sup>1</sup> the finding of sporophores of *Polyporus berkeleyi* attached to the roots of the larch in Montana, but from observations made in that region reached the conclusion that the mycelium ramified in the deep forest litter and drew its food from that source as well as from the rotten roots to which the sporophores were attached. It will prove very interesting if this rot in the larch should prove to be similar to that produced by this fungus in the oak, especially since the anatomical character of the wood of these trees is so different.

#### A STRAW-COLORED ROT OF OAKS CAUSED BY POLYPORUS FRONDOSUS

The initial stage of the straw-colored rot of the white oak (*Quercus alba*) is characterized by the dissolution of the middle lamellæ and the delignification of some of the wood fibers, leaving the fibers now consisting of cellulose free from each other. (Pl. VIII, fig. 4.) The advancing line of the rot upward in the tree consists of irregular, rather indefinite white patches, conforming more or less in size and shape to the largest medullary rays, or of narrow white bands projecting into the sound wood. Five or six inches below the boundary line between the advancing rot and sound wood the color in radial sections is more evenly white, as the patches have coalesced more or less at this stage. The unpolished split surface feels velvety, owing to numerous projecting free ends of the cellulose fibers. A tangential view of the advancing line of rot shows a whitish surface consisting of white delignified fibers interspersed with unchanged medullary rays and unchanged or only partially delignified vessels and their immediate adjacent tissue. In cross section the rot has a whitish cast surrounded by the natural color of sound heartwood.

The amount of delignified tissues in the earlier stages of this rot is much less than that found in the earlier stages of the string and ray rot. Eight to twelve inches behind the advancing point of the rot numerous colorless hyphæ are found in the larger vessels. At this stage in the rot some of the delignified tissue has been entirely absorbed. The delignification and absorption begin with the inner layer of the wood fibers and proceed centrifugally, so that the lumen of the cell

<sup>1</sup> Weir, J. R. Some observations on *Polyporus berkeleyi*. *Phytopathology*, v. 3, no. 2, p. 101-103, pl. 9, 1913.

gradually increases in size as the rot progresses. Marked delignification occurs in the tracheids and cells immediately adjacent to the larger vessels in which the fungous hyphæ are found, but the medullary rays and walls of the large vessels are still strongly lignified, as are also the numerous tyloses seen in these vessels. The walls of the tyloses were punctured in many places by the fungous hyphæ. Six to eight inches farther down, or 18 to 24 inches behind the advancing line of the rot, the rotted wood is soft and spongy to the touch and is of a straw color. In this stage the rotted wood consists of partially changed medullary rays, some unchanged wood fibers, and vessels with fragments of these in various stages of absorption, all strongly permeated with fungous hyphæ. Some medullary rays are still intact, while others have their outer radial cells either partially or entirely delignified and absorbed, so that in pulling apart the rotted wood tangentially, the medullary rays often pull out, leaving holes in one piece similar in size and shape to the rays, while the rays themselves remain attached to the other piece of the rotted wood.

The final stage of the rot differs but little from this condition, since there are still portions of all the elements present either unchanged or only partially changed. The rotted wood is rather tough and can be bent and twisted without breaking if taken in pieces 12 to 18 inches long and 4 or 6 inches thick. It is rather soft and spongy, but the fungus apparently never completely disorganizes the wood, thereby producing hollows. On weathering for two or three months the rot in the tops of the stumps and in the ends of the rejected butt cuts turns reddish brown and becomes firmly agglutinated, a condition so characteristic of this rot that one could identify the rot by this feature alone, without the presence of sporophores.

The rot has no odor. A section through the reddish discolored wood shows an abundance of light-brown hyphæ. The remnants of the remaining lignified tissues are also colored light brown. In a freshly cut stump which had this rot it would be hard to identify the rot in a cross section. Even when the wood is split open, there are no very pronounced macroscopic characters to distinguish it, like the string and ray stage of the rot caused by *Polyporus berkeleyi*.

The following is a brief description of the gross appearance of this rot caused by *Polyporus frondosus*, made as soon as the tree was cut.

The rot seen in a radial longitudinal view consisted of long white lines advancing 6 to 10 inches beyond the more completely rotted wood below. These lines apparently were caused by the fungous hyphæ following the vessels in certain annual rings. There was a watery reddish discoloration or "soak" about 2 inches in advance of the white lines. The older rot was of a light-tan or straw color and with a slight mycelial web in checks. Some 2 to 6 inches below the upper end of the white lines, white downy masses of mycelium could be seen by the aid of a hand lens in the large spring vessels situated in the white lines. In cross section the rot had a

coarse, fibrous surface, due to the stiff unabsorbed ends of the vessels, partially isolated by the absorption of the wood fibers and the subsequent tearing apart by the saw when the tree was felled. This fibrous character was not evident except where the tree was sawed.

This rot was identified in only 12 trees out of the 1,968 white oaks examined. No idea was obtained as to its age in a tree, as all of the trees found affected by it had open scars at their bases. It was apparently through such scars that the fungus entered the tree. Sporophores of *Polyporus frondosus* were found attached to the roots of 6 of the trees in much the same manner as those of *P. berkeleyi*, usually on that side of the tree which had the fire scar. The average height in the 12 trees attacked was 2.3 feet, the average diameter of the rot 12 inches from the ground was 14 inches, and the average age of the trees attacked was 270 years. The minimum age of the trees attacked was 220 years and the maximum age was 340 years. The maximum diameter of the rot in a tree was 24 inches and the maximum height in the tree was 4 feet.

The only external evidence of this rot in a tree was the presence of the sporophores attached to the roots of the diseased tree. The connection between the attached sporophores and the heart-rot in the tree was easily established in every case. This fungus may not continue to grow in the diseased trees after they are cut, for no sporophores have been found on felled trees nor have any been reported as occurring on logs. It seems to be strictly a butt-rot, as no evidence is known to the writer of its occurrence in the tops or on the branches of trees. One tree was found in which this rot was associated with the rot produced by *Hydnum erinaceus*. The writer has also found sporophores of *P. frondosus* on the roots of *Quercus digitata* at Arlington, Va., and has examined authentic herbarium material of the plant on the following hosts and from the following localities: "In evergreen woods," Canada; "under oak," Massachusetts; "at base of oak," Massachusetts; "at base of red oak," New York; from Ohio, no host given; "on old stump," Ohio; "at roots of fallen oak," Ohio; "at roots of oak," Maryland; "on dead trunks, 'Aceris negri,'" Missouri; "on roots of chestnut," Germany; "on roots of chestnut," Italy; "on *Castanea vesca*," France; "at base of large oaks," Saxony; "at base of trunk," Italy; and "on roots of chestnut," Bohemia (?).

This fungus, which has been known to mycologists for many years, is represented in nearly all the more complete lists of European fungi. It is evidently very widely distributed, inhabiting frondose woods in North America and Europe, in direct association with oak and chestnut trees.

The writer is under many obligations to the officers in charge of the New York Botanical Garden for the many courtesies extended to him while there, and to Dr. W. G. Farlow for free access to the Cryptogamic Herbarium of Harvard University.

## DESCRIPTION OF PLATES

**PLATE VII.** Fig. 1.—*Polyporus pilotae*: A sporophore on the end of a white-oak log from Arkansas. Photograph made 43 days after tree was felled.

Fig. 2.—*Polyporus pilotae*: Rot appearing in the butt of a white-oak log from Arkansas, showing the holes and white cellulose areas characteristic of this rot in a cross section of a living oak.

Fig. 3.—*Polyporus pilotae*: Radial-longitudinal view of a white-oak log from Arkansas, showing the honeycomb type of the rot with the white cellulose lines and elliptical hollows.

Fig. 4.—*Polyporus pilotae*: Rot occurring in a log of *Castanea pumila* from Arkansas; A, concentric layers of the rotted wood; B, white cellulose fibers.

Fig. 5.—*Polyporus pilotae*: Cross section of a chestnut log from New York, showing the central circular rotted zone.

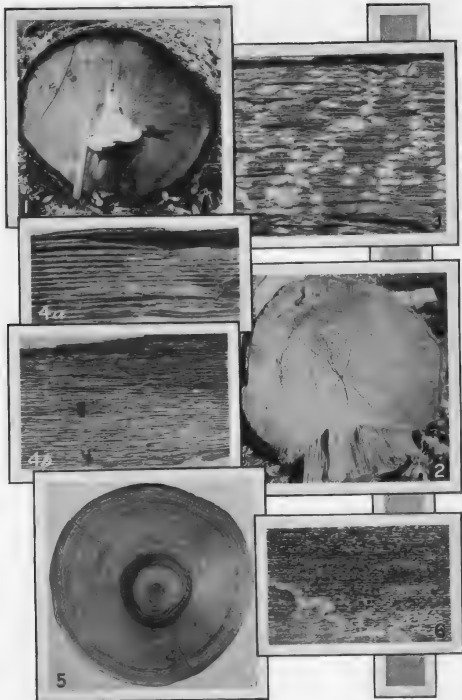
Fig. 6.—*Polyporus pilotae*: Radial-longitudinal view of the rot in a chestnut log from New York, showing the white pocketed stage.

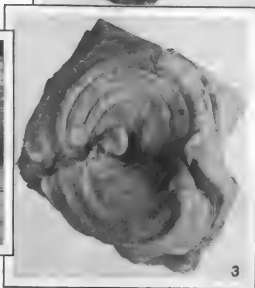
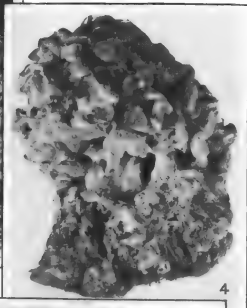
**VIII.** Fig. 1.—*Polyporus pilotae*: Radial-longitudinal view of the rot in a chestnut log from New York. This rot enters at a dead branch and then moves down the heartwood of the branch into the trunk.

Fig. 2.—*Polyporus berkeleyi*: Radial-longitudinal view of the rot in white-oak timber from Arkansas, showing the string and ray form characteristic of its second stage.

Fig. 3.—*Polyporus berkeleyi*: A sporophore on a white-oak root from Arkansas.

Fig. 4.—*Polyporus frondosus*: A sporophore on roots of white oak from Arkansas.





# INDIVIDUAL VARIATION IN THE ALKALOIDAL CONTENT OF BELLADONNA PLANTS

By ARTHUR F. SIEVERS,

*Chemical Biologist, Office of Drug-Plant and Poisonous-Plant Investigations, Bureau of Plant Industry*

## INTRODUCTION

It has long been recognized that a necessity exists for the improvement of the important medicinal plants. Within recent years agricultural science has been largely concerned with the improvement of crops by the application of the methods of plant breeding, but thus far practically no attempts have been made to extend these methods to drug plants with a view to improving their medicinal qualities. The chief aim in applying such methods should be to increase the active medicinal constituents rather than to improve the appearance of the plants. That the amount of a chemical constituent in a plant can be favorably modified by selection has been amply proved by work which has been done on the sugar beet, and there is reason to believe, therefore, that similar efforts with the economically important medicinal plants will be attended with success.

One of the first steps necessary to inaugurate such a plan is to determine the variation of the active constituents in individual plants and the extent to which such variation is influenced, if at all, by the various factors affecting the growth and cultivation of the plants. This article deals entirely with such a study. The results herein set forth furnish a basis for the application of the principle of selection as the next step in the solution of the problem.

*Atropa belladonna* was selected as a suitable plant with which to work, since it is probably the most important of the group of solanaceous plants which depend for their therapeutic action on mydriatic alkaloids. The supply of this plant in the wild state is largely exhausted and future supplies must necessarily depend on cultivation. The alkaloids which it contains can be definitely determined by chemical assay, which is a distinct advantage in a problem of this kind.

The writer wishes to emphasize the fact that the work thus far done constitutes but a preliminary step toward the application of the methods of selective breeding, which has already been begun. Considerable interest attaches to the results presented in this article because they represent the first extensive study of the variation of the quantity of alkaloids in this important economic plant.



VARIATIONS IN THE ALKALOIDAL CONTENT OF LEAVES OF DIFFERENT  
BELLADONNA PLANTS

## METHOD OF INVESTIGATION

The object of this investigation was to study the variation in the alkaloidal content of the leaves of individual belladonna plants and to establish, if possible, some correlation between the appearance of the plant and the variation of active constituents, should any variation exist. It was decided that the plants to be used for this purpose should be selected entirely at random, and they were therefore taken from all sections of the plat without reference to size or thriftiness. This afforded an opportunity to study the relationship of growth to alkaloidal content of the leaves. The field work was carried on at Arlington, Va., Bell, Md., and Madison, Wis. The Arlington plat was the largest, and the large number of plants at that place furnished the most complete data.

The general plan followed was to pick the leaves from each plant at different times during the growing season so as to be able to determine the proper time of the year in which the leaves should be picked in order to insure the greatest percentage of alkaloids. This should have the further advantage of showing whether individual plants which contain an abnormally high or low percentage of alkaloids in the leaves at one time of the year possess the same feature at other times.

Unfortunately this program could not be followed the first year, owing to pressure of other work. In some cases, especially early in the season, the plants were too small to furnish sufficient leaves for an assay without being entirely denuded. The smallest amount of dry material that could be used in the assay was 2 grams, and in order to insure a duplicate assay it would be necessary to have at least 25 grams of green leaves. Immediately after picking, the leaves were spread out evenly on a table in a dry, well-ventilated room until air-dry. They were then placed in small cloth bags until assayed.

The development of this investigation has been somewhat retarded through the loss of a number of the plants under observation. The loss was especially severe in the lower section of the plat, where the drainage is poor. The plants wilted suddenly and rapidly and the roots became entirely decayed. The loss was greatest after a prolonged wet spell, and after the trouble had once manifested itself the plants only occasionally recovered. Holmes<sup>1</sup> says that the cultivation of belladonna can rarely be continued beyond the third year, as the increased weight of the plants has a tendency to split the roots, thus permitting the water to enter and rot them. This may possibly be the trouble encountered here, but there is little evidence to show that the weight of the plant or mechanical injury is responsible, as both young and old plants suffered from the trouble.

---

<sup>1</sup> Holmes, E. M. The cultivation of medicinal plants in Lincolnshire. *Pharm. Jour.*, s. 3, v. 12, Sept. 17, p. 237-239, 1881.

## RESULTS OF THREE YEARS' OBSERVATION

During the summer of 1909 three rows of belladonna plants were started at the Arlington Experimental Farm directly from field sowing. The plants made a fair growth in the late summer and fall, but did not bear seed. The following spring they made a good growth and 24 plants were carefully staked out for this investigation. Since the plants made only a partial growth in the preceding year, they were considered as first-year plants and are so referred to throughout this article. The only picking from these plants in the first year was made in June, when most of the plants were in full bloom, although some were bearing berries of considerable size. Table I shows the general physical condition of the plants and gives the percentage of alkaloids in the leaves of each individual plant.

TABLE I.—Description of individual first-year belladonna plants and percentage of alkaloids in the leaves of each at Arlington Experimental Farm in June, 1910.

Plant No.	Stage of growth.	Description of plant.			Remarks.	Alkaloids.
		Height.	Spread.	Number of stems.		
		<i>Inches.</i>	<i>Feet.</i>			<i>Per cent.</i>
1	Not yet flowering....	12	1 by 1½	1	.....	. 645
2	Few flowers.....	18	2 by 2½	5	.....	. 618
3	.....do.....	12	3½ by 2½	8	.....	. 528
4	Slightly past flowering.	24	1½ by 3	5	.....	. 495
5	Flowers and some berries.	18	1 by 3	4	.....	. 334
6	.....do.....	12	3 by 3¼	4	Squatty.....	. 459
7	.....do.....	24	2½ by 3½	4	Tall.....	. 667
8	Many flowers.....	21	1½ by 3	3	One branch tall and erect.	. 657
9	Flowers and a few berries.	21	1½ by 4	2	.....	. 563
10	Many flowers.....	42	3½ by 4½	2	.....	. 536
11	Flowers and berries..	18	3 by 3¾	5	.....	. 587
12	.....do.....	15	2 by 3¾	3	.....	. 603
13	.....do.....	21	3½ by 4¼	8	.....	. 700
14	.....do.....	21	3 by 3	12	.....	. 656
15	.....do.....	24	3 by 4	8	.....	. 555
16	.....do.....	24	1½ by 3½	2	.....	. 544
17	.....do.....	15	2½ by 4	3	.....	. 485
18	Flowers and a few berries.	24	2 by 2¼	5	Very backward in development.	. 462
19	Flowers.....	12	1½ by 1¾	3	.....	. 440
20	Not yet flowering....	15	1½ by 2	1	Poorly developed...	. 473
21	.....do.....	18	1 by 1½	1	.....do.....	. 587
22	Flowers and berries..	12	2 by 4	3	.....	. 623
23	Not yet flowering....	12	1¼ by 1½	1	Poorly developed...	. 412
24	Flowers.....	18	1¼ by 1½	1	.....	. 503
	Average.....	.....	.....	.....	.....	. 547

Since one picking showed such a wide range of variation among the 24 plants, 35 additional plants were staked out the following spring. Twenty-six of these were in the same plat as the first plants, while the remaining 9 were in a neighboring plat on practically the same kind of soil and were separated from the others by a space of only about 100 feet. These 9 plants are distinguished in Table II by the letter "w."

Table II represents the results of the second and third years. In 1911, five pickings were made, extending from May 12 to October 17. At each picking the height of each plant was measured, until the full stage of development had been reached. At the first picking, on May 12, nine of the plants were not sufficiently advanced to furnish samples of leaves. Some of the more advanced plants were beginning to have flowers. On May 22, when the second picking was made, the plants were all in the full flowering stage. The third picking was made on June 17, when the flowering was mostly over and the berries generally were well developed. The plants had made considerable growth since the previous picking, but by the latter part of June they had reached their maximum growth. At the time of the fourth picking, September 6, they had assumed their characteristic late-summer and fall appearance. The berries were ripe and the leaves were small and sparse. At this stage the picking of the leaves is a very tedious process. Later in the fall, after the berries are ripe, new leaves begin to appear on the plant. Many of them develop on the new sprouts which mature during the summer, and not a few appear as the result of suckers which sprout directly from the roots. It has frequently been observed that some plants develop so many of these suckers that they have the appearance of plants just before flowering. At this stage, October 17, the fifth and last picking was made.

TABLE II.—Description of belladonna plants and percentage of alkaloids in the leaves of each at different stages of growth in 1911 and 1912.

Plant No.	Season of 1911.										Season of 1912.											
	Description of plant.					Alkaloids (per cent).					Description of plant.					Alkaloids (per cent).						
	Num-ber of stems.	Height (inches).			June 17.	First picking (May 12).	Second picking (May 22).	Third picking (June 17).	Fourth picking (Sept. 6).	Fifth picking (Oct. 17).	Aver- age for season.	Num-ber of stems.	Height (inches).			June 18.	First picking (May 10).	Second picking (May 21).	Third picking (June 18).	Fourth picking (Sept. 10).	Fifth picking (Oct. 17).	Aver- age for season.
		May 12.	May 22.	June 17.									May 9.	May 21.								
I	1	3	4	12	.....	.....	.....	0.823	0.687	0.755	0.501	1	10	15	22	.....	0.332	0.503	0.653	0.417	0.501	
2	5	6	15	24	0.852	0.698	0.583	.804	.619	.711	.568	3	19	16	23	.....	.558	.700	.407	.606	.568	
3	5	12	15	28	.384	.375	.277	.549	.451	.407	.429	5	12	16	19	.....	.....	.393	.448	.....	.429	
4	12	18	30	40	.461	.334	.304	.824	.665	.518	.559	13	30	34	36	0.522	.557	.469	.654	.591	.559	
5	3	8	16	24	.493	.700	.478	.681	.546	.579	.474	4	12	16	20	.....	.....	.438	.510	.....	.474	
6	4	10	18	28	.484	.....	.371	.630	.610	.524	.472	9	21	22	30	.462	.415	.....	.548	.465	.472	
7	5	9	20	42	.714	.622	.440	.765	.470	.602	.464	5	21	28	32	.....	.424	.447	.689	.296	.464	
8	4	8	16	30	.520	.618	.423	.891	.528	.596	.689	2	12	17	20	.....	.....	.652	.726	.....	.689	
9	2	6	12	20	.....	.627	.460	.781	.452	.580	.429	3	14	22	20	.560	.384	.402	.501	.....	.429	
10	7	13	22	38	.382	.344	.450	.671	.670	.593	.420	13	33	40	43	.....	.421	.438	.457	.....	.420	
11	7	8	17	33	.699	.677	.567	.537	.618	.619	.499	4	14	19	21	.....	.388	.609	.692	.223	.499	
12	3	15	30	39	.649	.782	.693	.497	.584	.641	.638	6	20	25	30	.767	.631	.685	.715	.308	.638	
13	9	18	32	42	.614	.627	.626	.763	.563	.639	.518	10	28	32	34	.495	.479	.655	.472	.394	.518	
14	10	9	24	38	.480	.556	.474	.476	.455	.488	.420	15	30	33	36	.553	.421	.457	.359	.488	.420	
15	4	16	30	45	.452	.....	.562	.546	.418	.494	.550	9	34	36	44	.641	.531	.567	.462	.310	.550	
16	5	14	22	36	.455	.754	.624	.622	.592	.609	.631	8	17	22	32	.716	.631	.545	.....	.....	.631	
17	7	12	24	30	.487	.....	.534	.396	.474	.473	.611	14	32	34	37	.684	.591	.559	.....	.....	.611	
18	10	11	22	30	.477	.645	.481	.634	.519	.551	.534	9	27	32	31	.553	.386	.662	.....	.....	.534	
19	6	6	15	30	.356	.386	.356	.597	.532	.427	.486	18	27	30	37	.560	.423	.475	.....	.....	.486	
20	2	6	16	24	.....	.489	.795	.599	.479	.568	.678	7	20	20	25	.....	.676	.681	.....	.....	.678	
21	3	6	12	22	.....	.535	.633	.669	.684	.630	.744	5	25	34	34	.732	.719	.781	.....	.....	.744	
22	4	12	22	30	.423	.410	.428	.454	.557	.454	.586	7	25	30	30	.703	.593	.462	.....	.....	.586	
23	4	7	16	28	.....	.348	.354	.487	.425	.403	.401	8	21	26	36	.496	.366	.341	.....	.....	.401	
24	4	5	11	24	.....	.349	.392	.....	.....	.370	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
25	3	8	20	33	.....	.277	.335	.....	.....	.306	.489	6	20	28	35	.521	.438	.508	.....	.....	.489	
26	4	11	22	35	.....	.378	.527	.797	.733	.597	.726	12	25	30	36	.869	.700	.609	.....	.....	.726	

27	2	9	18	30	.287	.432	.554	.495	.442	8	28	29	35	.754	.693	.521	.....	.696
28	3	12	20	30	.392	.513	.740	.614	.513	9	27	31	35	.657	.535	.525	.....	.572
29	2	8	21	30	.655	.914	.968	.547	.756	5	22	25	31	.737	.647	.729	.....	.704
30	7	10	17	30	.387	.512	.653	.527	.530	9	24	25	29	.655	.553	.507	.....	.572
31	5	12	20	33	.381	.458	.669	.384	.464	1	3	7	7	.....	.....	.....	.....	.....
32	5	13	20	32	.409	.546	.703	.549	.526	7	24	27	31	.533	.446	.646	.....	.542
33	12	13	23	36	.481	.549	.496	.458	.486	5	22	25	28	.602	.589	.576	.....	.589
34	4	12	22	33	.335	.526	.532	.200	.414	4	19	23	26	.....	.521	.520	.....	.466
35	3	15	26	36	.323	.476	.708	.524	.430	7	24	31	40	.608	.596	.732	.590	.616
36	3	12	24	39	.320	.468	.488	.473	.446	6	27	33	38	.469	.418	.598	.555	.483
37	4	11	20	30	.323	.423	.719	.558	.515	6	22	30	33	.598	.442	.475	.705	.548
38	5	11	20	34	.532	.514	.749	.630	.595	8	23	28	34	.454	.463	.520	.586	.566
39	9	15	30	37	.303	.327	.614	.451	.391	6	25	30	36	.404	.365	.525	.600	.473
40	6	17	34	39	.405	.797	.797	.644	.560	15	26	34	35	.575	.556	.469	.....	.533
41	2	16	28	36	.318	.456	.562	.414	.428	7	29	32	36	.508	.512	.524	.470	.469
42	3	15	30	36	.494	.526	.615	.528	.533	4	24	28	34	.617	.664	.648	.789	.679
43	3	12	28	40	.515	.534	.547	.331	.499	4	19	26	30	.506	.365	.698	.562	.533
44	2	20	31	36	.342	.497	.626	.550	.489	3	25	27	26	.458	.....	.467	.....	.463
45	6	15	30	40	.467	.410	.759	.379	.488	11	24	30	38	.601	.501	.382	.430	.460
46	5	13	26	40	.337	.308	.588	.431	.390	9	21	34	30	.418	.334	.480	.483	.466
47	5	14	26	36	.389	.422	.573	.482	.453	6	27	32	38	.....	.479	.572	.449	.595
48	4	14	24	36	.494	.493	.748	.....	.568	3	21	28	30	.426	.346	.590	.567	.452
49	5	17	27	42	.583	.476	.668	.505	.558	7	21	25	26	.556	.476	.457	.578	.481
50	8	10	19	30	.610	.583	.619	.379	.527	3	13	19	24	.....	.361	.328	.420	.353
1W	17	8	21	28	.638	.447	.758	.612	.682	8	12	20	28	.737	.642	.777	.....	.719
2W	7	12	28	36	.444	.614	.311	.518	.531	7	24	30	32	.573	.544	.503	.....	.560
5W	17	18	34	39	.464	.741	.638	.595	.584	18	28	39	42	.741	.602	.524	.866	.664
6W	8	19	33	39	.596	.925	.711	.722	.766	8	25	28	34	.847	.747	.882	.804	.768
7W	13	16	32	36	.558	.831	.727	.571	.704	15	36	37	35	.782	.666	.646	.694	.672
8W	9	9	25	30	.578	.538	.570	.481	.577	12	25	32	34	.634	.453	.280	.505	.468
9W	4	11	23	30	.487	.553	.425	.337	.514	5	16	25	28	.557	.429	.638	.....	.541
10W	6	14	26	26	.587	.763	.424	.424	.587	8	20	25	28	.751	.478	.505	.639	.610
11W	6	11	24	30	.425	.373	.479	.420	.444	3	19	26	30	.773	.562	.430	.402	.544
Average..	.....	.....	.....	.....	.472	.517	.633	.519	.532	.....	.....	.....	.....	.601	.503	.553	.568	.545

In 1912 the same line of observation was followed in connection with the same plants and the results are also included in Table II. Unfortunately, the disease described elsewhere killed fully one-half of the plants by the end of the season. Therefore, the results given in the table are not as complete as those of the previous year, especially with regard to the fourth and fifth pickings. The stages of growth at which the pickings were made correspond closely to those of the previous year, as the dates indicate.

At the drug-testing garden at Bell, Md., where the soil is quite different from that at Arlington Experimental Farm, 19 individual plants were under observation and three pickings of leaves were made. Owing to a delay, no picking was made at the time of the first picking at Arlington, although the plants at both places were at the same stage of development. Consequently, the picking on May 27, which is designated as the first at Bell, corresponds to the second picking of the Arlington plants. Table III shows the results.

TABLE III.—*Description of individual belladonna plants and percentage of alkaloids in the leaves of each at different stages of growth, at Bell, Md., in 1911.*

Plant No.	Description of plant.			Alkaloids (per cent).			
	Number of stems.	Height (inches).		First picking May 27.	Second picking June 22.	Third picking Oct. 17.	Average for season.
		May 27.	June 22.				
1	5	22	22	0.329	0.288	0.422	0.346
2	4	19	22	.474	.502	.395	.457
3	8	20	24	.485	.408	.641	.511
4	4	23	26	.639	.686	.570	.632
5	3	24	24	.659	.637	.415	.570
6	2	25	24	.577	.637	.559	.624
7	4	23	24	.654	.722	.482	.619
8	3	25	24	.467	.464	.....	.466
9	3	24	26	.526	.595	.350	.477
10	6	23	26	.752	.600	.418	.590
11	4	15	18	.571	.485	.579	.545
12	5	26	28	.548	.....	.424	.486
13	5	22	24	.695	.587	.750	.677
14	5	27	30	.407	.605	.....	.506
15	3	16	18	.436	.....	.448	.442
16	5	30	28	.466	.390	.511	.456
17	7	28	30	.823	.665	.527	.675
18	6	33	34	.754	.689	.502	.648
19	6	24	22	.782	.783	.556	.707

At the drug-testing garden at Madison, Wis., observations similar to those at Arlington have been made for two years, and the results are given in Table IV. The first nine plants were under observation in 1911 and 1912, while the last eight were sent to Madison from Arlington as young seedlings in the spring of 1912. No notes were taken of the individual plants with regard to height, spread, and number of stems, since they were all very much alike. Each plant acquired a height of about 2 feet and had an average of three or four stems each.

The stages of growth at which these pickings were made correspond closely to the first, second, and third pickings at Arlington, irrespective of the dates. Since Madison is farther north than Washington, the plants came up later in spring than in the vicinity of Washington and did not reach the full flowering stage until in July or early in August.



TABLE IV.—Description of individual belladonna plants and percentage of alkaloids in the leaves of each at different stages of growth at Madison, Wis., in 1911 and 1912.

Plant No.	Season of 1911.										Season of 1912.										Average alkaloids for two seasons.	Per ct.
	General appearance and size of plant. <sup>1</sup>					Alkaloids (per cent).					General appearance and size of plant.					Alkaloids (per cent).						
	Appearance.	Height.	Spread.	Stems.	June 20.	July 5.	July 31.	Average for season.	Appearance.	Height.	Spread.	Stems.	June 24.	July 13.	July 15.	Aug. 7.	Average for season.					
1 <sup>2</sup>	Excellent.	In. 18	In. 18	2	0.566	0.548	0.767	0.627	Excellent.	In.	In.											
2	Good.	12	12	4	.496	.478	.528	.500	Good.	30	36	8	0.351	0.519			0.435					
3	do.	16	18	2	.580	.501	.520	.533	do.	30	24	4	.268	.501			.385					
4	Poor.	8	12	3	.492	.427	.420	.446	do.	30	30	6	.392	.417			.405					
5	Excellent.	18	18	3	.493	.545	.508	.515	Excellent.	42	48	13	.299	.445			.372					
6	do.	18	18	3	.418	.466	.664	.516	do.	39	48	11	.307	.316			.414					
7	Fair.	12	18	2	.....	.442	.419	.430	Fair.	27	24	5	.507	.534			.521					
8	Poor.	12	18	1	.558	.539	.485	.527	do.	18	18	7	.500	.404			.452					
9 <sup>3</sup>	do.	8	16	2	.561	.820	.615	.665									.489					
Average					.521	.529	.547	.529					.375	.448			.411					
10																						
11															0.323	0.397	.360					
12															.360	.496	.428					
13															.350	.412	.381					
14															.416	.408	.412					
15															.311	.497	.404					
16															.261	.252	.257					
17															.305	.378	.342					
Average															.409	.508	.459					
															.342	.418	.380					

<sup>1</sup> These data were taken at the time the last picking was made. By the end of the growing season, October 15, the plants had greatly increased in spread and also somewhat in height. This was due largely to the new fall growth.

<sup>2</sup> In 1912 this plant did not appear above ground until August 1.

<sup>3</sup> This plant was dead in 1912.

## RELATION OF THE ALKALOIDAL CONTENT OF THE LEAVES TO THE STAGE OF GROWTH OF THE PLANT

Opinions have been expressed from time to time as to the proper stage in the growth of the belladonna plant at which the leaves should be picked in order to insure the greatest percentage of alkaloids. Owing to the standard required by the Pharmacopœia, this is a question of no small economic importance. Gerrard<sup>1</sup> has found that the plant is not rich in alkaloids before flowering, but that the full development is reached at the period of flowering and is maintained in both the roots and leaves into the fruiting season.

The large number of assays of the leaves of individual plants here involved presents exceptional opportunity for the study of the above question. The proper season for the picking of belladonna leaves does not, however, depend entirely on the percentage of active constituents present. This will become very evident when the data at hand are thoroughly interpreted. Table V shows in condensed form the number of plants in which there was an increase or decrease in the percentage of alkaloids in the leaves at the various pickings.

TABLE V.—*Number of belladonna plants which showed an increase or decrease in percentage of alkaloids in the leaves at the second, third, fourth, and fifth pickings as compared with the preceding picking at Arlington Experimental Farm in 1911 and 1912.*

Stage of growth.	Season of 1911.			Season of 1912.		
	Total number of plants.	Number of plants which showed—		Total number of plants.	Number of plants which showed—	
		Increase.	Decrease.		Increase.	Decrease.
Second picking.....	70	38	32	59	16	43
Third picking.....	60	25	35	53	34	29
Fourth picking.....	54	40	14	32	20	12
Fifth picking.....	56	8	48	23	4	19

Table V shows that in 1911 the leaves of most of the plants were richer in alkaloids at the second picking than at the first, which is in accord with the observations of Gerrard, already noted. In 1912, however, the opposite is true. It will be seen further that in the fourth picking of both years the greatest number of plants showed an increase in the alkaloidal content of their leaves. Referring to Table II, it is seen that in the fourth picking in 1911 the average quantity of alkaloids for the leaves of all the Arlington plants was 0.633 per cent, or more than one-tenth of 1 per cent than at the flowering stage. In 1912, at

<sup>1</sup> Gerrard, A. W. On the alkaloidal value of belladonna plants at different periods of growth. Year-book of Pharmacy, 1881-1882, p. 400-404, 1882.

this same stage, the average was 0.568 per cent of alkaloids, which is 0.065 per cent higher than the average at the flowering stage, although lower in this case than at the early stage. There appears to be but a slight difference so far as the alkaloidal content is concerned between the flowering stage and the early fruiting stage. At the last, or fifth, picking, the plants had acquired much new growth and, judging from the average results, the percentage of alkaloids present in the leaves at that stage was not much different from the second and third stages.

Although the experiments show that the leaves are richest in alkaloids at the late fruiting stage of the plant, collection at that time for commercial purposes is practically out of the question because the leaves are of very small size. After the flowering period is over and the berries are ripening many of the large leaves fall off and numerous small, bractlike leaves develop. These, while apparently rich in alkaloids, could not be picked to advantage in large quantities.

#### RELATION OF SIZE AND APPEARANCE OF PLANTS TO ALKALOIDAL CONTENT OF LEAVES

When this investigation was first undertaken it was hoped that some relationship might be found to exist between the physical appearance of the plants and the alkaloidal content of their leaves, for should such relationship exist the process of distinguishing between the good and the poor plants with regard to their active constituents would become a much simpler matter than by use of the assay method, since the latter is necessarily tedious.

The variations in the physical appearance of belladonna plants depend largely on the height and the number of stalks or stems. When height is referred to here, the actual length of the stems from the ground to the tips is meant rather than the vertical distance of the topmost branches from the ground. This distinction is necessary because many of the branches droop or grow at an angle. The spread of the plant, that is, the distance around, is largely dependent upon the angles at which the branches are growing and on the number of stems of the plant. The height of the plant and the number of stems, therefore, are the two distinguishing features as regards size. These indicate also the relative health and vigor of the plant. An attempt was made to differentiate between various types of leaves, with reference to size and color and between different types as regards blooming and fruiting tendencies. It was found difficult, however, to find individuals which conformed definitely to any particular type. Where certain characteristics existed they were not as a rule general over the entire plant, but were usually found on only one side or on only certain stems. Thus, in some cases, one or two stems of a plant bore what appeared to be leaves of a larger size than usual and of a different shade of green,

while the remainder of the plant was in every respect like most of the other plants. The same would be true of the number of flowers and berries. In such cases it could not be assumed that the plant represented any special type. It was also noticed that some of these distinctive features were subject to gradual changes, so that their distinctiveness was soon lost.

While the number of plants that have been under observation was probably not sufficiently large to show conclusively that there is no definite correlation between physical appearance and active medicinal properties in the leaves, yet from the data at hand such a condition is at least indicated. Henderson,<sup>1</sup> in commenting on the great variation in the alkaloidal content of different lots of belladonna roots, points out that appearance is no criterion of the quality, the best appearing roots being often the poorest in medicinal value.

To show by actual examples that there is apparently no relation between the appearance of the plant and its alkaloidal content it is necessary only to refer to the tables. For example, in Table I plant No. 10 has a height of 42 inches and a spread of  $3\frac{1}{2}$  by  $4\frac{1}{2}$  feet; in fact, it is the largest plant in the list, yet its leaves contain only 0.536 per cent of alkaloids, which is a trifle less than the average of all the plants. On the other hand, plant No. 8, which is only half as high and much smaller in spread, shows 0.657 per cent of alkaloids in its leaves. Again, in Table II (season of 1911) plant No. 15 is the largest in the plot in point of height, yet its leaves assayed only 0.494 per cent, or less than the average quantity of alkaloids. A similar statement may be made in regard to large plants Nos. 4, 43, 45, and 46, while, on the other hand, the leaves of the comparatively small plants, Nos. 21, 29, and 17, contained 0.630, 0.756, and 0.682 per cent of alkaloids, respectively. The data show that in the following year these same plants failed again to compare favorably with others as regards size, yet the percentages of active constituent in their leaves stand out prominently above the average. However, plants can be pointed out in the same table which are larger and apparently more vigorous than the average and which also contain above the average percentage of alkaloids in their leaves. The lack of correlation is therefore very evident.

#### VARIATION AMONG PLANTS

Among the facts brought out by this investigation probably the most important is the great variation in the percentage of alkaloids found in the leaves of individual plants at each of the three testing gardens. That some variation should exist was to be expected, since variations are often noted in the chemical constituents of different plants of many

<sup>1</sup> Henderson, H. J. Percentage of alkaloid in belladonna root. *Pharm. Jour.*, v. 75, no. 3485 (S. 4, v. 21, no. 1832), p. 191, 1905.

other species. The knowledge of the existence of such individual variations should have an important bearing on the question of the improvement of drug plants by selection and cultivation.

To show the great variation found among the comparatively limited number of plants under observation Table VI is here presented.

TABLE VI.—*Range of variation in percentage of alkaloids in the leaves of belladonna plants at each stage of growth, at Arlington, Madison, and Bell stations, in different years.*

Stage of growth.	Alkaloidal content of the leaves (per cent).											
	Arlington, Va.						Madison, Wis.				Bell, Md.	
	1910.		1911.		1912.		1911.		1912.		1911.	
	High.	Low.	High.	Low.	High.	Low.	High.	Low.	High.	Low.	High.	Low.
First picking.....			0.852	0.303	0.869	0.404	0.580	0.418	0.500	0.268	0.823	0.329
Second picking.....	0.700	0.334	.879	.262	.747	.292	.820	.427	.519	.316	.783	.288
Third picking.....			.925	.277	.882	.328	.767	.419			.750	.395
Fourth picking.....			.891	.311	.806	.359						
Fifth picking.....			.733	.200	.678	.296						
Season average.....			.766	.306	.768	.353	.665	.430	.452	.312	.707	.346
Average.....			.841	.277	.792	.339	.708	.423	.490	.298	.766	.339

From this tabulation it appears that the active principle is more than three times as great in the leaves of some plants as in those of others at the same period of growth, although the plants are in the same plat and therefore grow practically in the same soil and under the same climatic conditions. Under such circumstances the existing variation can hardly be attributed to anything but the inherent characteristic of the individual plant. Much has been written concerning the influence of soil and climate on the formation of alkaloids in the plants. Gerrard<sup>1</sup> has found that a chalky soil favors the formation of atropin. Chevalier<sup>2</sup> concludes from his experiments with fertilizers that the alkaloidal content of certain Solanaceæ can be increased by means of nitrates and farmyard manures. Ransom and Henderson,<sup>3</sup> however, who are working along the line of Chevalier's experiment, have not found thus far that artificial manures materially affect the percentage of alkaloids in the dried leaf, but note in several cases a large increase in the yield of the

<sup>1</sup> Gerrard, A. W. Op. cit.

<sup>2</sup> Chevalier, J. Influence de la culture sur la teneur en alcaloïdes de quelques Solanées. *Compt. Rend. Acad. Sci. (Paris)*, t. 150, p. 344-346, 1910.

<sup>3</sup> Ransom, Francis, and Henderson, H. J. Belladonna: the effects of cultivation and fertilizers on the growth of the plant and its alkaloidal content. *Chemist and Druggist*, v. 81, no. 1703, p. 53-55, 1912.

green plant per acre. Carr<sup>1</sup> claims to have found a certain relationship between the amount of sunshine during the growth of the plant and the percentage of alkaloids found in the stems and leaves, claiming that plenty of sunshine and limited rainfall have a tendency to stimulate the production of alkaloids.

Although soil and climate may have considerable influence on the alkaloidal content of plants, yet to establish this as a fact beyond all doubt is a difficult matter because of the individual variation involved. Until experiments have been conducted upon a large number of plants which show a minimum variation in their alkaloidal content, nothing definite can be said upon this point. In working with a limited number of plants collectively, an abnormally low or high percentage of alkaloids in the leaves of a few might so affect the yield as to make the average entirely misleading. Likewise, this individual variation becomes an important matter in the sampling of large quantities of leaves and roots. In order to secure a reliable sample, it should be of considerable size and selected only after the leaves or roots have been thoroughly mixed.

#### INDIVIDUAL VARIATION THROUGH SEVERAL SEASONS

Having definitely established the fact that great variations exist in the alkaloidal content of the leaves of individual plants, the question remains to be answered whether such variations exist only during one growing season or whether they manifest themselves in the same proportion in following seasons. If plants which are rich in alkaloids one season are correspondingly poor the following season, then it is logical to assume that the production of alkaloids in the plant is dependent on factors which change from year to year. If it were definitely known what rôle the alkaloids play in the metabolism of the plant, it might be easier to determine what factors influence their development. As has been shown, the physical appearance, or, in other words, the vitality and growing power of the plant, appears to bear no definite relation to the development of alkaloids. Furthermore, if soil and climate are the potent factors, then their influence ought to be felt by all plants alike when all are grown on similar soil and in the same locality. Such, however, has been found not to be the case, and reference to the tables shows that there were plants rich and poor in alkaloids in every year during which the observations extended. On the other hand, if it should be found that a plant with leaves containing an unusually high or low percentage of alkaloids in one season shows the same characteristics in following years, it would be safe to assume that there is a definite tendency in that plant to produce a small or a large quantity of alkaloids in the

<sup>1</sup> Carr, F. H. The effect of cultivation upon the alkaloidal content of *Atropa belladonna*. *Chemist and Druggist*, v. 81, no. 1703, p. 42-44, 1912.

course of a season's growth, just as in other plants there are well-defined tendencies toward certain physical characteristics.

This investigation, however, has hardly progressed far enough to yield any definite conclusions. In Table VII a comparison is made between

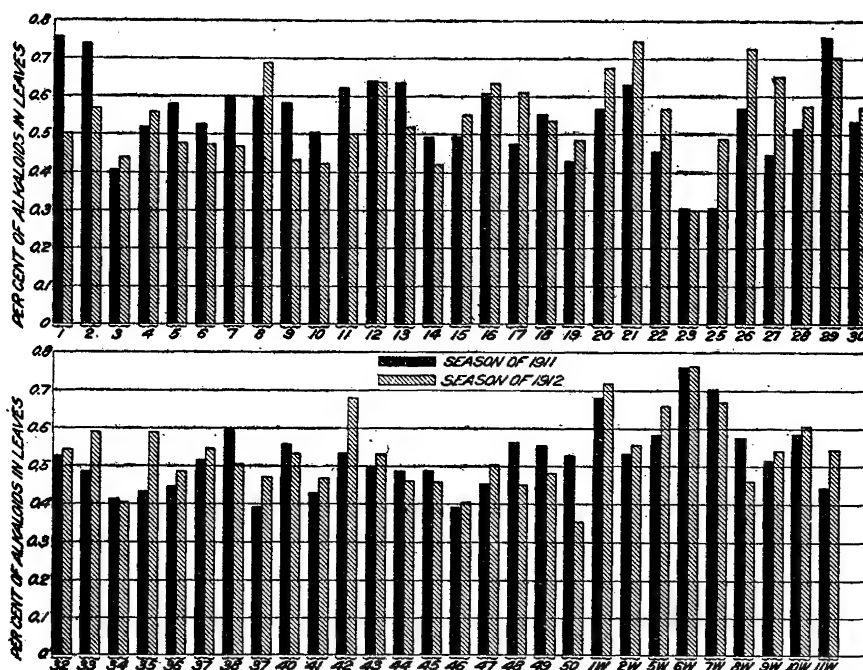


FIG. 1.—Diagram showing the percentage of alkaloids in the leaves of individual belladonna plants at the Arlington Experimental Farm, Va., during the seasons of 1911 and 1912.

the years 1911 and 1912 of the 59 plants grown at Arlington, showing the variation of alkaloidal content above and below the average for each of the years mentioned. Figure 1 shows graphically the seasonal comparison.

TABLE VII.—Percentage of alkaloids above and below the average<sup>1</sup> in the leaves of individual belladonna plants at Arlington, Va., in 1911 and 1912.

[The figures given are based on the season averages of all the pickings. In each of the 40 plants designated by a star (\*) the percentage of alkaloids above or below the average of the entire lot in 1911 varies by not more than one-tenth of 1 per cent from that in 1912.]

Plant No.	Alkaloids above (+) or below (—) the average (per cent).		Plant No.	Alkaloids above (+) or below (—) the average (per cent).	
	1911.	1912.		1911.	1912.
1.....	+0.223	—0.044	31.....	—0.068	.....
2.....	+ .179	+ .023	32*.....	— .006	—0.003
3*.....	— .125	— .116	33*.....	— .046	+ .044
4*.....	— .014	+ .014	34*.....	— .118	— .139
5.....	+ .047	— .071	35.....	— .102	+ .071
6*.....	— .008	— .073	36*.....	— .086	— .062
7.....	+ .070	— .081	37*.....	— .017	+ .003
8*.....	+ .064	+ .144	38*.....	+ .063	+ .061
9.....	+ .048	— .116	39*.....	— .141	— .072
10*.....	— .029	— .125	40*.....	+ .028	— .012
11.....	+ .087	— .055	41*.....	— .104	— .076
12*.....	+ .109	+ .093	42.....	+ .001	+ .134
13.....	+ .107	— .023	43*.....	— .033	— .012
14*.....	— .044	— .125	44*.....	— .043	— .082
15*.....	— .038	+ .005	45*.....	— .044	— .085
16*.....	+ .077	+ .086	46*.....	— .142	— .139
17.....	— .059	+ .066	47*.....	— .079	— .040
18*.....	+ .019	— .011	48.....	+ .036	— .093
19*.....	— .005	— .059	49*.....	+ .024	— .064
20*.....	+ .036	+ .133	50.....	— .005	— .192
21*.....	+ .098	+ .199	1w*.....	+ .150	+ .174
22.....	— .078	+ .041	2w*.....	— .001	+ .015
23*.....	— .129	— .144	5w*.....	+ .052	+ .081
24.....	— .162	.....	6w*.....	+ .234	+ .243
25.....	— .226	— .056	7w*.....	+ .172	+ .127
26.....	+ .035	+ .181	8w.....	+ .045	— .077
27.....	— .090	+ .111	9w*.....	— .018	— .004
28*.....	— .019	+ .027	10w*.....	+ .055	+ .065
29*.....	+ .224	+ .159	11w*.....	— .088	— .001
30*.....	— .002	+ .027			

<sup>1</sup> Average for 1911, 0.532 per cent; for 1912, 0.545 per cent.

In the plants in Table VII there are a number which are conspicuous because of the high or low percentage of alkaloids in their leaves. Plants Nos. 3, 23, 34, and 46 are without doubt greatly inferior to the others from a medicinal point of view. On the other hand, Nos. 21, 29, 1w, 6w, and 7w are greatly superior to any others in the list. Furthermore, these plants manifested the same characteristics not only on the average but at each picking. The recapitulation given in Table VIII shows this very clearly.



TABLE VIII.—*Alkaloidal content of the leaves of belladonna plants, rich and poor in alkaloids, at various stages of growth, in 1911 and 1912.*

Stage of growth (picking).	Plants with leaves of low alkaloidal content (per cent).							
	No. 3.		No. 23.		No. 34.		No. 46.	
	1911.	1912.	1911.	1912.	1911.	1912.	1911.	1912.
First.....	0.384	.....	.....	0.496	0.335	.....	0.337	0.418
Second.....	.375	0.393	0.348	.366	.....	0.292	.285	.334
Third.....	.277	.448	.354	.341	.526	.520	.308	.480
Fourth.....	.549	.448	.487	.....	.532	.....	.588	.483
Fifth.....	.451	.....	.425	.....	.200	.....	.431	.314
Average.....	.407	.429	.403	.401	.414	.406	.390	.406

Stage of growth (picking).	Plants with leaves of high alkaloidal content (per cent).									
	No. 21.		No. 29.		No. 1W.		No. 6W.		No. 7W.	
	1911.	1912.	1911.	1912.	1911.	1912.	1911.	1912.	1911.	1912.
First.....	.....	0.732	.....	0.737	0.638	0.737	0.596	0.847	0.558	0.782
Second.....	0.535	.719	0.655	.647	.835	.642	.879	.747	.831	.666
Third.....	.633	.781	.914	.729	.587	.777	.925	.882	.832	.646
Fourth.....	.669	.....	.908	.....	.738	.....	.711	.804	.727	.694
Fifth.....	.684	.....	.547	.....	.612	.....	.722	.558	.571	.573
Average.....	.630	.744	.756	.704	.682	.719	.766	.768	.704	.672

## SUMMARY

From the point of view of the percentage of alkaloids present in the leaves and the quantity of material available, the leaves can be picked to best advantage from the time of flowering until the early berries begin to ripen. Although the leaves are richer in alkaloids later in the season, they are then too small and sparse for harvesting.

Thus far nothing has been found to indicate that any correlation exists between the physical appearance of the plant and the alkaloidal content of its leaves. Luxuriant growth is by no means a criterion of the medicinal value of the plant.

The variation of the percentage of alkaloids in the leaves of the different plants is exceedingly large. This makes it a difficult matter to determine to what extent soil and climate influence the development of alkaloids. Where such wide variations exist among individual plants, the testing of a general sample from all plants collectively is not always a safe means of judgment.

A considerable number of plants with leaves rich in alkaloids in one season are found to have equally rich leaves in the following season. Furthermore, they frequently manifest the same characteristics at the various stages of growth during the season in comparison with other plants. The same facts are true with regard to plants which bear leaves with a low percentage of alkaloids.

# THE PUBESCENT-FRUITED SPECIES OF PRUNUS OF THE SOUTHWESTERN STATES

By SILAS C. MASON,  
*Arboriculturist, Crop Physiology and Breeding Investigations, Bureau of Plant Industry*

## INTRODUCTION

The species of the genus *Prunus* described in this article occupy a unique position in the flora of the western United States from the fact that their relationship with the wild plums of the country is remote and they are more closely allied to some of the Asiatic species of this genus.

Their economic importance arises chiefly from their close adaptation to the climatic and soil conditions of the Southwest, where fluctuations of heat and cold, severe drought, and considerable alkalinity of the soil must be endured by most tree crops.

Adaptable stocks for the cultivated forms of *Prunus* capable of meeting such conditions are eagerly sought. Species with such characters which are capable of being hybridized with the old-established cultivated forms of the genus offer attractive possibilities to the plant breeder. This is especially true of the one edible-fruited form, *Prunus texana*, which affords in aroma and flavor of fruit most attractive characters for combination with other stone fruits of larger size and more staple commercial character.

Instead of forming a homogeneous group, as has usually been believed, these species fall into small groups of quite diverse character and affinities. To the plant breeder and student of their economic possibilities these relationships are of such importance that the following detailed study of them is deemed essential to an intelligent use of them in plant-breeding work.

In parts of the country beyond the Rocky Mountains a few ranchmen, occasionally a solitary mining prospector, and a few local botanists know of curious bushy plants growing in desert wastes having plumlike bark and twigs, oddly shaped leaves, and small downy fruits with thin dry flesh which have won for them the local names "wild almond" in the Great Basin region, "wild peach" or "desert almond" for another form in the Mohave Desert, and "wild apricot" or "wild almond" for a third form in the foothills bordering the Salton Basin in southern California.

A fourth form has been known for many years to the pioneers of eastern Texas, who have enjoyed eating the "wild peach" of their sandy

country, the only really edible fruit of the group. However, this fruit is still strangely ignored by horticulturists and botanists alike.

A fifth form, growing in the limestone plains of central Texas, has a dry and inedible fruit which has not sufficiently attracted the attention of the cattlemen and goat herders in this sparsely settled region to earn a local name.

A sixth form, growing in the high altitudes of both northern and southern Mexico, though the first of all to receive botanical notice (1823) is still very rare in herbaria and has been seen in its native habitat by but few botanical explorers. It was first collected by Humboldt in his famous journey through the Mexican plateau region. A seventh species, Havard's wild almond, still very imperfectly known, has recently been described from the region inclosed by the Big Bend of the Rio Grande in western Texas.

We have, then, native to the region of North America, lying west of the Mississippi drainage area, six or seven members of the plum family differing in a very marked way from the familiar types of American wild plums.

They are united by the common character of a woolly or pubescent fruit, and all are deep-rooted, with remarkable drought resistance. This fruit character, so at variance<sup>1</sup> with the true plums of America or of the Old World, would at first seem to ally these species with the almond or apricot sections of the genus, as their common names suggest. A close examination of their botanical characters shows, however, that they fail to agree with those groups and must be regarded as occupying intermediate ground between the true plums on the one hand and the almonds or apricots on the other. Aside from the common character of pubescent fruit and their deep-rooting habit, these species differ widely from one another, which is to be expected from the wide geographic range which they occupy and the resulting differences in climate and soil.

#### HABITAT AND ENVIRONMENT

Ranging farthest north is the commonly named "wild almond" (*Prunus andersonii*), which is found around the shores of Pyramid Lake, Nev., in the Honey Lake region of California, and along the basin slopes of the Sierras, having an altitude range of from 4,000 to 8,000 feet in the Upper Sonoran and Transition life zones. (See map, fig. 1.) This is consequently subject to severe cold in winter, as much as 20° F. below zero in some instances, and to extreme drought and severe heat in the summer. It is usually found in gravelly or sandy soils.

Its near relative, the "wild apricot" (*Prunus eriogyna*), found along the desert slope of the San Bernardino and Santa Rosa Mountains and

<sup>1</sup> *Prunus oregana* Greene, of Oregon and northern California, has fruit with a fine, soft pubescence, but it is a true plum, near to *P. subcordata*.

southward into Lower California, is an inhabitant of much lower altitudes, at least in California. There it occurs at from 500 to 3,000 feet in the upper margin of the Lower Sonoran, but chiefly in the Upper Sonoran zone, extending a little below the zone of light winter snow, though subject to intense heat and prolonged drought in summer. (See map, fig. 1.)

Most similar to this species in habitat and requirements, though remote in relationship, is the "desert almond"<sup>1</sup> (*Prunus fasciculata*). This fruit

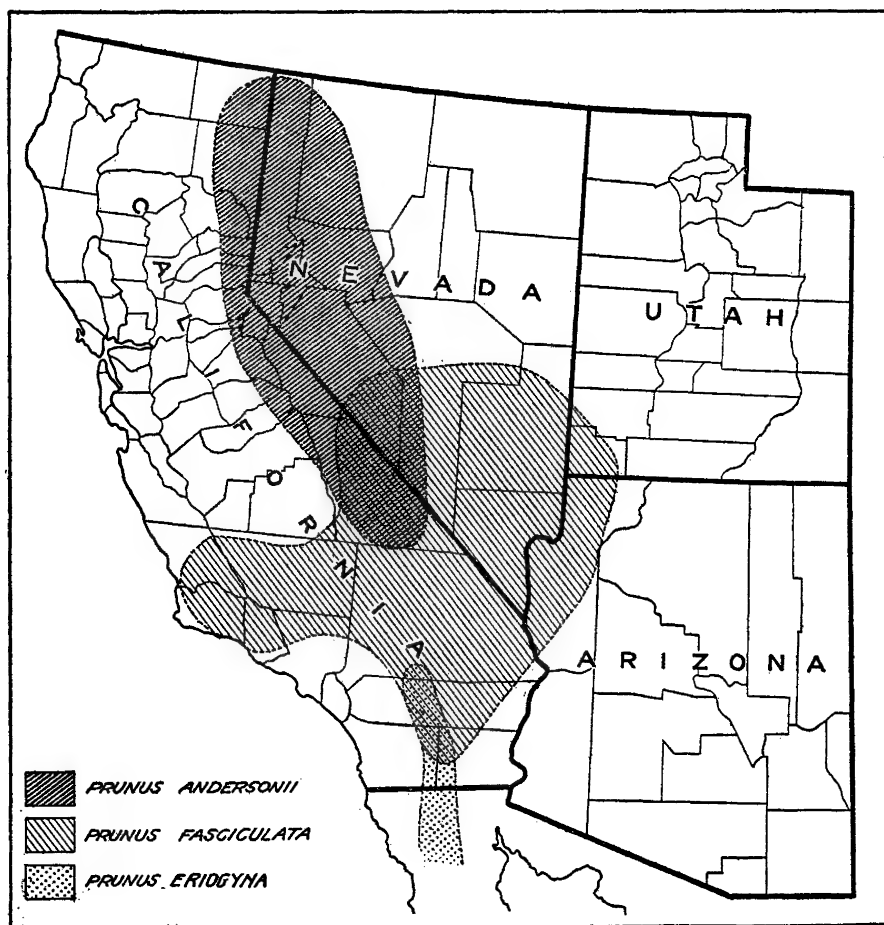


FIG. 1.—Map of the southwestern part of the United States, showing the range of *Prunus andersonii*, *Prunus fasciculata*, and *Prunus eriogyna*, n. sp.

occurs in widely scattered localities over a range which includes southern Nevada and California, together with the adjacent portions of Utah and Arizona. It overlaps portions of the areas of both *Prunus andersonii* and *Prunus eriogyna*, but, like the latter, is found in the upper margin of the Lower Sonoran and in the Upper Sonoran zones. (See map, fig. 1.) It

<sup>1</sup> Called "desert range almond" by Dr. C. H. Merriam in notes on the distribution of trees and shrubs in the deserts . . . U. S. Dept. Agr., Bur. Biol. Survey, North American Fauna, no. 7, p. 301, 1893.

is nowhere subject to the severe cold endured by the Nevada "wild almond" in its most northern habitat. It usually grows in gravelly formations or along washes or sandy slopes where deep root penetration is possible.

This "desert almond" is remote geographically from the two species of the group to which it is most nearly allied, the Texas wild almond, *Prunus minutiflora*, and its Mexican cousin, *Prunus microphylla*, which may consistently be called the "Mexican wild almond."

The Texas species has a range not yet well worked out, but it is apparently confined to the Cretaceous limestone region of the southwestern portion of the State, extending across the Rio Grande into the State of Chihuahua, Mexico, and probably occurring in Coahuila. Its known localities are entirely in the upper portion of the Lower Sonoran zone. (Fig. 2.) It is found over an area ranging in altitude from 750 feet near San Antonio to 3,000 feet near the mouth of the Pecos River, with an average rainfall of about 20 inches, but subject to periods of prolonged drought. There is an absolute temperature range for the years recorded of from zero to 110° F., with the liability to sudden drops from winter northers, peculiar to this region.

Of the conditions under which the Mexican species grows we have but indefinite knowledge, but it occurs at high altitudes—6,000 to 8,000 feet, the Upper Sonoran zone of this southern latitude, probably a mild temperate climate with light winter rains and heavy summer showers. In common with the other species it grows in a region where the setting of the fruit is frequently prevented by late spring frosts.

The little-known Havard's wild almond, *Prunus havardii*, apparently a near relative of these two species last mentioned, has been found so far only in western Texas.

The Texas "wild peach," *Prunus texana*, occurs in scattered localities over a region of eastern Texas from near sea level to nearly 2,000 feet in elevation, lying wholly in the Lower Sonoran or Lower Austral zones. This includes a portion of the western extremities of the corn and cotton belts, where an apparently sufficient annual rainfall is so unevenly distributed that long periods of drought make agriculture somewhat precarious and render irrigation a needful adjunct. It is adjacent to the area of *Prunus minutiflora*, but the division with its sharp demarcation is not one of climate, but of soils. *Prunus minutiflora* follows the Cretaceous limestone of the plateau region, while *Prunus texana* occurs on the mellow granitic sandy soil of the "Burnet Country" or the sandy loam of the Coastal Plain and is wholly wanting on limestone soils. (See map, fig. 2.)

# BOTANICAL CHARACTERS OF THE GROUP

The botanical characters of the seven species under consideration, even the obvious character of the leaves and fruit, are so distinct from those generally recognized as belonging to wild or cultivated plums that it is not surprising that the Mohave Desert form was first assigned by Dr. Torrey to a new genus, *Emplectocladus*, from the Greek words referring to its interlocking branches. This was later placed in the genus *Prunus* by Gray, but as a separate section. Schneider, while including all these

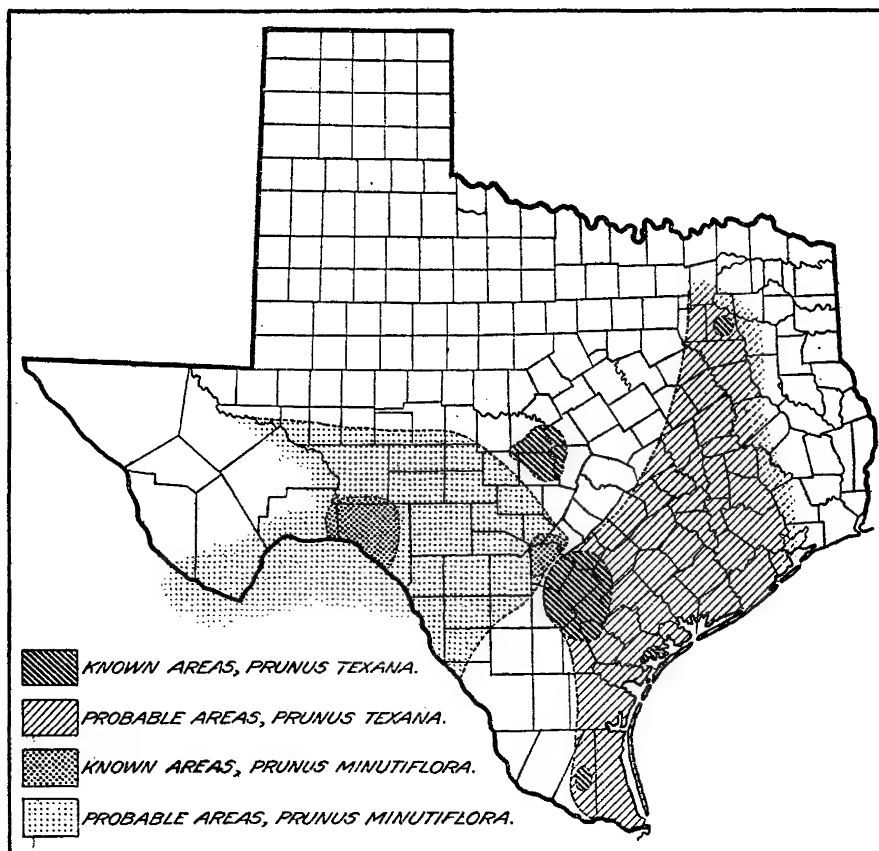


FIG. 2.—Map of Texas, showing the known areas and probable range of *Prunus minutiflora* and *Prunus texana*.

species under *Prunus*,<sup>1</sup> groups them in the section *Emplectocladus* along with Torrey's original species, *Prunus (Emplectocladus) fasciculata*, and the Old World *P. pedunculata*. Several authors have assigned some or all of the species to *Amygdalus*.

The study of the entire group from abundant material and the field examination of all but *Prunus microphylla* and *P. havardii* convince the writer that they are separable into three distinct sections.

<sup>1</sup> Schneider, C. K. *Illustriertes Handbuch der Laubholzkunde*. Bd. 1, Lfg. 4, Jena, 1905, p. 589-590.

The so-called wild almond (*Prunus andersonii*), chiefly found in Nevada, though also occurring along the eastern slope of the Sierras in California, is upon careful comparison found to be very closely related to the wild apricot (*Prunus eriogyna*) of the Colorado Desert in southern California. These two species are clearly separated from the peach and almond by the characters of the leaves both in veneration and when mature, by floral characters, and by the seeds.

The entire group (the genus *Amygdalus* of some authors) of the genus *Prunus* which includes the almonds and peaches has leaves folded lengthwise in the bud (conduplicate), the flowers sessile or subsessile, the stones rugose and pitted.

The Nevada wild almond, notwithstanding the fact that it has been described as being "a true almond in its affinities,"<sup>1</sup> and the desert apricot agree with the section *Armeniaca*, the apricots, in three important points: First, the leaves in the bud are rolled from the margin toward the middle, or convolute; second, the flowers are stalked, some on pedicels three-fourths of an inch long; and, third, the stones are smooth or but faintly pitted and decidedly wing-margined.

These characters are found also in some of the true plums, but a distinct separation from the plums is met in the rose-colored flowers and in the only slightly fleshy, pubescent fruits.

The presence of stomates in the upper surfaces of the leaves is a character distinguishing these two species from both the *Amygdalus* and *Armeniaca* sections.

Their characters as a whole, however, seem to unite them most closely with the apricots, and apparently there is nothing among the European and Asiatic forms of *Prunus* to which they are as closely related. Consequently the two species are here placed in a new section, *Penarmeniaca* (near-apricots).

The California desert almond (*Prunus fasciculata*), the Texas wild almond (*P. minutiflora*), and the Mexican wild almond (*P. microphylla*), agree in three important characters which separate them clearly from the three other species of this group. All three are dioecious by the abortion of either stamens or pistils; the number of the stamens is usually reduced to 10 or 15 and a portion of them inserted on the walls of the calyx cup. They further agree in having the inner face of the cup finely hairy instead of having a nectariferous surface as in apricots, peaches, and almonds. Havard's wild almond probably belongs in this same group.

*Prunus fasciculata* has leaves with stomates in the upper surface, in which it resembles *P. andersonii* and *P. eriogyna*, while the other three species have no stomates in the upper surface. However, on the strength of the characters possessed in common, especially of the remarkable one of the dioecious character of the flowers, *Prunus fasciculata* is placed with

<sup>1</sup> Greene, E. L. *Flora Franciscana*. [Pt. 1], San Francisco, [1891] p. 49.

the Texas and Mexican wild almonds in the subgenus *Emplectocladus* of *Prunus*. This has been done with a full realization that most definitions of this genus describe the flowers as perfect, though Sargent<sup>1</sup> and Schneider extend the definition to include polygamo-dioecious flowers. No reference to dioecious or polygamo-dioecious characters in any Asiatic forms of *Prunus* has been found.

While a more complete knowledge of the Asiatic forms<sup>2</sup> may disclose closer affinities for these three species, they are retained provisionally as the sole member of the subgenus *Emplectocladus*. With our present knowledge of these forms the seven species of *Prunus* studied in this paper should be grouped as follows:

#### SCHEME OF CLASSIFICATION

##### PRUNUS

##### SUBGENUS EMPECTOCLADUS

Low divaricate or erect shrubs with more or less spinescent branches. Bark on new growth gray or brownish, glabrous or more or less pubescent. Leaves conduplicate in veneration; borne singly on vigorous young growth or apparently fascicled on budlike suppressed branchlet, with or without stomates in upper epidermis.

Flowers solitary or gemminate, sometimes crowded on short fruiting spurs, subsessile, precocious or coetaneous with the leaves, dioecious by the abortion of stamens or pistils; calyx cup obconic or campanulate, glabrous or faintly puberulous on the outer surface, minutely hairy within; stamens usually 10 to 15 on short filaments, in three more or less well-defined circles, inserted on the margin of the cup and on the walls below; ovary and base of style pubescent.

Fruit seldom more than 1 cm. long, pubescent, subglobose or irregularly ovate, with thin, dry flesh splitting tardily, and smooth or obscurely ridged stone.

Four species: *Prunus fasciculata* Gray, *Prunus minutiflora* Engelm., *Prunus microphylla* Hems., and *Prunus havardii* (Wight), n. comb.

##### SUBGENUS EUPRUNUS

##### SECTION PILOPRUNUS, N. SECT.

Low, much branched, often procumbent, scarcely spinescent shrubs, with gray or brown, pubescent young wood.

Leaves conduplicate, without stomates in upper epidermis, tomentose, glandular serrate.

<sup>1</sup> Sargent, C. S. *Silva of North America*. Boston, 1892, v. 4, p. 7.

<sup>2</sup> *Prunus pedunculata* (Pall.) Maxim. and *P. pilosa* (Turcz.) Maxim. of Mongolia are said by Koehne (Pl. Wilsonianæ, pt. 2, p. 273) to have the calyx cup dry within and minutely hairy at the insertion of the stamens. Schneider figures (Laubhk., v. 1, p. 598, fig. 335<sup>a</sup>) the whole interior of the calyx cup of *P. pedunculata* as finely hairy. Little is known as to the flower characters of *Prunus boissierii* Carr. from Asia Minor referred to *P. pedunculata* by Schneider, but which differs in having sessile flowers. These plants are referred to the section *Emplectocladus* by Schneider, but his figures of *P. pedunculata* show a perfect flower and no hint is given in descriptions of the other forms of their flowers being dioecious. These species, as well as the little-known *P. mongolica* and *P. dehiscens* Koch., grouped along with them by Koehne (Pl. Wilsonianæ, pt. 2, p. 274), and *P. betunnikowi* Litw. doubtfully referred to this group by Schneider (Laubhk., v. 2, p. 974), all need to be studied carefully so as to permit of a careful comparison with the American forms here referred to the section *Emplectocladus*.



Flowers white, appearing with the leaves, fascicled on short pubescent peduncles, perfect, highly fragrant; calyx cup campanulate, pubescent without, nectariferous within, with glandular serrate lobes; ovary finely pubescent.

Fruit 1.5 cm. to 2.5 cm. long, pubescent, the juicy, fragrant, highly flavored flesh clinging to the stone by a persistent velvety pile; stone rounded, smooth or scarcely furrowed.

One species: *Prunus texana* Dietr.

#### SECTION PENARMENIACA, N. SECT.

Dense shrubs with angled and thorny branches or of more smooth and erect arborescent growth reaching 3 meters in height; young twigs glabrous, reddish or yellow brown.

Leaves convolute in vernation, glabrous, more or less glandular serrate, with stomates in the upper epidermis.

Flowers rose colored, pale pink, or rarely white, solitary or in fascicles of two or three, on stalks from 5 to 15 mm. in length; stamens 20 or 30, inserted near the rim of the calyx cup; calyx cup campanulate, with nectariferous lining; pistil as long or longer than the stamens; ovary and base of style pubescent.

Fruit oval or subglobose, 1 to 2 cm. long, pubescent, somewhat fleshy while immature, harsh and astringent but with an acid, fruity flavor, opening along suture when mature; stone thick walled, furrowed, with obscure reticulations or smooth or somewhat pitted; kernel in some varieties edible, often strongly flavored with prussic acid.

Two species: *Prunus andersonii* Gray and *Prunus eriogyna*, n. sp.

#### THE WILD PEACH

The earlier botanical descriptions of the important species *Prunus texana* are so meager that the following description in greater detail seems necessary:

##### *Prunus texana* Dietr.<sup>1</sup>

*Amygdalus glandulosa* Hooker, Icon. Pl., v. 3, pl. 288, 1840.

*Prunus glandulosa* (Hooker) Torr. and Gray, Fl. N. A., v. 1, p. 408, 1840.

*Prunus texana* Dietr., Syn. pl., v. 3, p. 45, 1843.

*Prunus Hookeri* Schneider, Laubhk., v. 1, Lfg. 5, p. 597-598, fig. 335, i, k, l, 1906.

*Amygdalus texana* (Dietr.) W. F. Wight, Dudley Mem. Vol., p. 131, 1913.

Illus. Hooker, loc. cit.; Schneider, loc. cit.

Low, squarrose shrubs, sometimes reaching a height of 2 meters, with a spread of 2 to 2.5 meters; stems usually slender but occasionally erect and stout branches, rarely spinescent; bark dark iron gray, roughly furrowed on old wood, on young growth grayish brown or silvery gray, densely pubescent.

The leaves, conduplicate in the bud, are usually narrowly elliptical, with rounded apex and rounded or wedge-shaped base; thick, strongly veined, serrate or crenately doubly serrate, with glandular teeth, dull green, thickly pubescent above, canescent beneath, 1.5 to 4 cm. long, 6 to 18 mm. broad; petiole short, rather thick, stipules 3 to 4 mm. long, narrowly lanceolate, with glandular teeth.

The small flowers, which appear with the leaves in February and March, are fragrant, perfect, 1 to 1.5 cm. broad, borne singly or in fascicles of two or three on short, finely pubescent peduncles; the campanulate calyx tube is finely pubescent,

<sup>1</sup> There being a *Prunus glandulosa* of Thunberg, 1784, Hooker's *Amygdalus glandulosa* can not be transferred to the genus *Prunus* and the name *Prunus texana*, given by David Dietrich (Synopsis plantarum, v. 3, Vimariae, 1843, p. 45), has priority and is a most appropriate one, as this interesting species has so far been found only within the limits of Texas. This conclusion as to the priority of Dietrich's specific name is confirmed and published by Dr. C. S. Sargent in Trees and Shrubs, v. 2, pt. 3, Boston, June, 1911.

the strongly reflexed, short rounded lobes being glandular ciliate margined, with fine soft hairs on both surfaces. The inner face of the tube is lined with an orange-colored, nectariferous layer. The thin white petals, 5 mm. long, are broadly ovate, often truncate at the base, attached by short, stout claws. The ovary and two-thirds of the length of the style are finely pubescent. The fruit is roundish oval or oblong, usually with a ventral shallow furrow, 1 to 2.5 cm. in length, a sharp depression at the base, pedicel 5 to 8 mm. long. The skin is rather thick, coated with fine pubescence, yellowish, greenish yellow, or rarely taking a rich reddish flush on one side; flesh yellowish or greenish yellow, finely netted, juicy and luscious, sometimes very richly flavored, clinging to the rather large stone by a curious tough, persistent elastic pile, like coarse plush, which, when scraped away, leaves an ovate obtusely pointed, thin-walled seed without pits or furrows. The kernel is plump, roundish pointed, slightly furrowed, and with a strong flavor of prussic acid. (Pl. IX, and fig. 3.)

It is plain that with its strongly glandular pubescent leaves and luscious, fleshy fruit with the pilose or velvety stone it has little near relationship with the five species of the group in which it has been included. It has accordingly been placed in the subgenus *Euprunus* and in a new section, *Piloprunus*. Analogy for the pubescent fruit is found in the *Prunus oregana* of Greene and for the netted flesh clinging to the stone in the sand plum, *P. watsoni*.

With a promising wild species of distinctly limited range it is of first importance to learn under what conditions of soil, temperature, and rainfall it has been able to reach its present standing in the plant world. In a State affording so vast an "open range," so to speak, as the State of Texas, the restriction of a species to a range must mean certain limitations in endurance. If it stops rather sharply as soil types change, with no other apparent reason for not extending farther in that direction, we must suspect a soil preference amounting to limitation. A fairly well-defined northern boundary is pretty sure to mark the limit of cold endurance, provided soil and moisture conditions would seem to invite farther advance in that direction. Therefore, the geographic range or distribution of the wild peach should be studied and also related conditions of soil, temperature, and moisture.

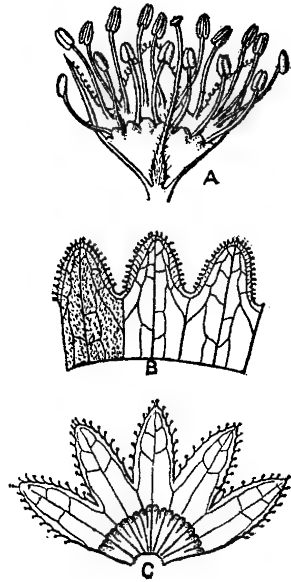


FIG. 3.—*Prunus texana* Dietr.: A, Section of calyx,  $\times 3$ ; B, detail of calyx lobes, showing glandular margins,  $\times 3$ ; C, section of calyx from flower of the horticultural variety Ramsey, *P. texana*  $\times$  Wild Goose plum,  $\times 4$ .

## DISTRIBUTION AND SOIL

The range of the wild peach is wholly within the State of Texas, but its local distribution is not yet worked out. As shown by the map (fig. 2), there are two principal areas of its growth. The first of these is what is called the "Burnet Country," a region of granitic uplift occupying the greater portion of Llano County, and small areas of Burnet, San Saba, Mason, Gillespie, and Blanco counties. It is also found along a narrow alluvial strip next to the Colorado River in Lampasas County.

It is upon the sedentary soils from granitic disintegration, small areas from sandstone and schistose rocks of the earlier stratified formations bordering and upturned by the granitic protrusions, and on narrow strips of river alluvium that the "wild peach" occurs. Only one instance is known of its occurrence upon the calcareous areas which surround and in isolated patches overlap the granitic protrusions.

The second considerable area known for this species lies in the southeastern part of Bexar County and in the adjacent counties of Guadalupe, Wilson, and Atascosa, extending eastward into Gonzales and southward into Bee County. As this region is a part of the area of sandstone formation known geologically as the Marine Eocene region and the plants are found only on rather mellow sandy soils, we must conclude that the species has so strong a preference for granitic or sandy soils as to practically exclude it from limestone regions. It was learned in the neighborhood of Lavernia that extensive areas of this "May plum," as it is called in that section, had been destroyed in the clearing up of fields. Isolated patches have been found at points as remotely separated as Van Zandt County at the north, the coast dunes of Aransas County, and a considerable area in the sandhills of Hidalgo County at the south, where the fruit is much esteemed by the Mexicans under the name "durasnillo," or "little peach."<sup>1</sup> It seems probable that a more complete survey of the eastern portions of the State would show that the wild peach has a botanical range extending over a greater portion of the sandy formation of the Marine Tertiary region, restricted probably by lack of moisture in the southwest portion of that formation.

All the plants studied have a deep-rooting habit, enabling them to penetrate to layers of soil where the moisture is fairly permanent as is often the case where the soil has a sandy foundation. This aids them greatly in surviving the long periods of drought to which the country is subject. The thickly pubescent upper surface of the leaves and the almost felted undersurface are features which reduce transpiration and must aid materially in drought resistance.

<sup>1</sup> Prof. S. W. Stanfield, of the Texas State Normal School, states that in southern Bexar County this fruit is called "albaricoque," which is the Spanish name for the apricot.

## CLIMATIC CONDITIONS

The principal areas occupied by *Prunus texana* are represented by fairly complete weather records at Menardville, Fredericksburg, and San Antonio<sup>1</sup> and by volunteer records at Burnet, Llano, and Lampasas.<sup>2</sup> These show that the mean annual rainfall ranges from 22.6 inches in the more westerly to 28 inches in the eastern and southern portions.

The monthly means show a fairly well-distributed rainfall throughout the year. December to March constitutes the drier period, with February as the driest month. The study of the monthly records of a number of years, however, shows that this section is subject to occasional heavy rainfalls almost torrential in character, as well as to periods of severe and prolonged drought. A study of the extremes of rainfall at San Antonio, a nearly central point in the range of this species, shows that during the driest year of the period covered by the record, 1885 to 1903, only 15.9 inches of rain fell, while the maximum record was 40.5 inches. The structural characters enabling *Prunus texana*, the wild peach, to endure these vicissitudes are important features to study.

The temperature conditions characteristic of this section are those of comparatively mild winters, minimum temperatures of 12° to 16° F. being matters of common record, with occasional winters showing minimum records of as low as -2° to -4° F.<sup>3</sup>

Minimum temperatures of 50° to 60° and maximum temperatures of 60° to 75° F. may be followed in a short time by a norther which will lower the temperature to near the zero point, or even below. The extreme maximum temperatures experienced in this section are from 100° to 105° F.

## NATURAL HYBRIDIZATION

One of the most striking characteristics of the wild peach is the readiness with which it hybridizes with the native and cultivated plums. This is proved by the occurrence of well-marked natural hybrids with the local wild plums in at least five widely separated localities within its range.

The occurrence of natural hybrids between species of plants is unusual and in many families rare or unknown. The integrity of our plant forms could not be preserved if indiscriminate natural hybridizing were a possibility.

Probably among trees and shrubs the most numerous examples of such hybrids are afforded by the oaks of the Mississippi Valley and the Western States, and a number of these have from time to time received definite

<sup>1</sup> Henry, A. J. Climatology of the United States. U. S. Dept. Agr., Weather Bur., Bulletin Q., p. 431-436, 1906.

<sup>2</sup> U. S. Dept. Agr., Weather, Bur., Climate and Crop Service, Texas Section, v. 1-5, 1897-1901.

<sup>3</sup> There was a record of -4.1° F., at Llano, Feb. 12, 1899. U. S. Dept. Agr., Weather Bur., Climate and Crop Service, Texas Section, Report, v. 3, no. 5, p. 5, 1899.

botanical description. A few wild grape hybrids are recorded in the writings of Dr. Engelmann.

Among plums a few definite natural hybrids of the wild species have been recognized, and the later judgment of Prof. Bailey on *Prunus hortulana*, described by him as a species, is that it is a group of varieties which are hybrids between *Prunus americana* and the southwestern species, *Prunus angustifolia*.<sup>1</sup>

On the whole, surprisingly few authentic hybrids have come into existence without the aid of man among uncultivated plants.

Examples of natural or unassisted hybridization among cultivated plants are somewhat more common, as though as a result of cultivation some of the safeguards which nature had established against the interbreeding of species had been broken down, but here again the sum total of such crosses is small.

These considerations make it the more interesting and significant when we find such a divergent form of plum as this so-called wild peach hybridizing so freely with the local forms with which it comes in contact.

How numerous these hybrids may actually be is only a matter of conjecture, and only a close survey of the entire region of occurrence of *Prunus texana* can disclose this. The detection of these at any stage of active growth is rendered comparatively easy by the strongly marked characters of this species. The narrow, pubescent, strongly glandular-serrate leaves, as well as the pubescent calyx cup with glandular-serrate lobes, added to the notable character of the pubescent fruit with its peculiar pile-covered stone, all help to render one of this class of hybrids conspicuous and unmistakable. Three of the more striking forms in three widely separated localities had been noticed and taken into cultivation years ago by observant ranchmen interested in fruit growing. Systematic search by the writer and other observers, though for only a few days and over a very limited area, disclosed the other eight recorded.

It is significant that in five out of seven of the most important regions where the wild peach is associated with the wild species of plums these spontaneous crosses have been found. In these same sections hybrid forms between the numerous species of true plums are rare or have not been detected. More conclusive evidence can hardly be offered that *Prunus texana* crosses with a number of species of the true plums with unusual readiness, far more readily than these species cross among themselves. It is on account of this fact and the promise which it holds out to enterprising plum breeders that it has been thought worth while to describe in rather minute detail a number of these natural hybrids, including several which as horticultural varieties have little or no value.

The first of these varieties was learned of during an exploring trip around Kingsland, Llano County, in March, 1910, and through the kind-

---

<sup>1</sup> Willard, S. D., and Bailey, L. H. Notes upon plums for western New York. New York Cornell Agr. Exp. Sta., Bul. 131, p. 170, 1897.

ness of Mr. Henry Smith the writer was shown a group of bushes located on the Smith ranch near the foot of Pack Saddle Mountain, about 6 miles from Kingsland. These had been known to a few settlers in the neighborhood for many years and the fruit had been carefully gathered on account of its value in making preserves and jam. The "Llano" variety, named and propagated for distribution by Mr. L. Miller, a nurseryman at Lampasas, was secured in this neighborhood and is so nearly identical with those seen on the Smith ranch that a separate description is scarcely necessary.

The next group was located near the south line of Llano County not far from the Llano and Fredericksburg Road. Mr. F. M. Ramsey had previously discovered a bush in this region which from its appearance he believed to be a hybrid of the wild peach and a plum. On a trip with the writer in search of this plant two more were found in the same neighborhood. These are described in succeeding pages under the names "Willow," "Sumlin," and "Holman." They are of considerable interest as botanical hybrids showing the potency of the species *Prunus texana* rather than for the quality of the fruit produced.

Having been informed that at Valley Springs, about 12 miles northwest of Llano, a farmer had wild peaches growing in his garden and that with cultivation they grew as large as plum trees, another group of hybrids was suspected. A visit was accordingly made to the farm of Mr. N. F. Gephart, an early settler in the Valley Springs district, in whose orchard several plum trees just ripening fruit were found to show undoubted evidence of *Prunus texana* origin. Yet three clearly distinct varieties could be detected. The two which are described as the "Gephart" and the "Johnson" are interlocking trees which Mr. Gephart states he found in a wild state in clearing the ground for the orchard more than 20 years ago.

The history of the Bolen variety, with two or three others in the garden, is rather obscure. Mr. Gephart states that there used to be a tree of this character, long since disappeared, on a near-by farm known as the Bolen place. He is of the impression that seeds from this original Bolen fruit were planted in his garden and produced one or more trees, which bore well for a number of years but are now dead. There are at present several trees very similar in character. Whether they are from sprouts of the original seedlings of the Bolen tree or from a second generation of seedlings Mr. Gephart is uncertain. Apparently the first cross of *Prunus texana* was the original tree on the Bolen farm, from which the seedlings in the Gephart garden originated.

The following year, 1911, information was received of "a plum with a skin like a peach" growing in an orchard near Lavernia, Wilson County, 20 miles southeast of San Antonio, and on a visit to that place two small trees were found on the farm of Mr. W. J. Stuart, who reported that he had found a little group or thicket of these trees in a draw when clearing

part of his farm. These two had been transplanted to his plum orchard and the others grubbed up. Though not perfectly identical, these trees—though small, they were perfect trees in form—are so similar as to make it superfluous to give separate descriptions. The more perfect of these was selected for description and named “Stuart” for the owner. (Pl. X, figs. 1 and 2.) Its fruit is among the best produced by hybrids. Two points worthy of note about this variety are that the flowering followed the opening of the leaves and that there is a tendency to suckering or root-sprouting.

A few days later, in a trip along the Hilderbrand Road in company with Mr. R. E. Blair, two more hybrid varieties were found in a field of the Whittaker Ranch. Both were small trees, evidently sprouted from an older growth broken down. The flowering season had passed and a small setting of half-grown fruits was noted. Later in the season Mr. Blair returned and found a few of these ripe, but a detailed description was not secured. It is a medium-sized, dull-red fruit of only fair quality. The variety near the fence on the pike road was designated as the Hilderbrand and the one in the field as the Whittaker. On a later trip Mr. Blair located another hybrid tree in the same neighborhood, a detailed description of which has not been secured.

It will be noticed that in the descriptions of these hybrids no attempt has been made to name other parents than the wild peach (*Prunus texana*), which dominates them all. There are characters which indicate that at least three other species as parents must be reckoned with. The northern portion of the range of this species is also the home of a number of species of typical American plums.

Prof. Sargent has but recently described two new species from this territory, and it is probable that others are pending. No less than eight species of *Prunus* of the plum class have been credited to this territory, several of which are with difficulty distinguished from each other. The hopelessness of determining the other parents of these hybrids is immediately apparent. We have no basis for more than a conjecture as to which direction the cross may have taken, whether *Prunus texana* furnished the pollen or was the pistillate parent.

The only hint we can get in this direction is from the work of Mr. Ramsey, referred to later. He made use of pollen from the Wild Goose plum, without removing the stamens from the flowers of the wild peach and secured four hybrid trees out of a number of fruits set on the protected branch. All of these four show Wild Goose characteristics in their flowers.

The grouping of a number of closely similar varieties, as in the case of the two Gephart trees, the Stuart group, and those on the Hilderbrand Road, suggests that a bush of wild peach may have received visits of bees carrying wild-plum pollen and that a number of fruits of this pollination germinated under or near the parent bush.

## DESCRIPTIONS OF HYBRIDS

***Prunus hortulana* (Wild Goose) × *texana*.**

Hort. var. Ramsey.

A rather ragged branched tree about 2 meters high, with yellowish brown pubescent twigs of new growth. Leaves ovate lanceolate, acuminate at apex, rounded or broadly cuneate at base, serrate or doubly crenulate serrate, with short glandular teeth; upper surface dull with scattered short hairs; lower surface grayish green, silvery tomentulose; petiole stouter than in most of the hybrids, 5 mm. to 10 mm. long, tomentose; stipules narrow, acute, glandular toothed.

The flowers, which appear before the leaves, about the middle of March, are white, about 8 mm. broad, borne in three or four flowered umbels on slender, pubescent pedicels. The calyx is pubescent, lobes pubescent on both surfaces, margins glandular.

The fruit, ripe about June 15, is globose, 2 to 2.5 cm. long, the rather thick dull-red skin sparingly tomentulose, the thin reddish flesh clinging to the velvety coated pit, which is turgid, oval, pointed at either end, and with a broad ventral ridge; the pedicels are 8 to 10 mm. long and stouter than in most of the hybrids of the species. This fruit is acid, rather austere, but of value in making jellies, marmalades, etc. The originator, Mr. Ramsey, states that it is a remarkably regular bearer. It seems to thrive well on a strongly calcareous soil and has been grown to a good size worked on peach stock.

***Prunus texana* hybrid.**

Hort. var. Llano.

A low, ragged bush, 1 to 1.5 meters high, as it occurs in thickets in the stony pastures in Llano County, where it was first observed more than 30 years ago and where it spreads slowly by means of root sprouts. Worked on peach stock the twigs of young growth become long, slender, and pendulous with little disposition to spiny branches. The twigs of young growth are reddish brown, thinly pubescent.

Leaves elliptical or ovate elliptical, apex acute or narrowed and shortly acuminate, base rounded or broadly cuneate; margin serrate or doubly serrate; the teeth glandular tipped; the upper surface dull green, with scattering short silvery hairs; russet green with thin pubescence beneath; 3.5 to 4 cm. long, 1.5 to 2 cm. broad; the midribs yellowish brown; slender petioles about 7 mm. long; stipules 3 to 5 mm. long, narrow, acute, coarsely glandular toothed. The flowers appearing with the leaves are white, 5 to 8 mm. broad; calyx tube campanulate, pubescent; lobes short, broadly ovate, with glandular teeth and hairy inner surface; petals obovate with short claw.

The fruit, ripening in June, is globose, a little compressed, 2 cm. in diameter; color dull red; skin rather thick, coated with a thin, fine pubescence; flesh netted, clinging to the pit, which is turgid; oval, obtuse at base and apex, coated with velvet pile; pedicel short. This fruit, produced in great abundance, is of a sharply acid flavor, but is highly esteemed for domestic use.

***Prunus texana* hybrid.**

Hort. var. Willow.

A willowy shrub, 1 meter high, profusely branched, the branches angled at nodes, long, slender, tapering; young growth greenish brown, pubescent, but becoming smooth iron gray with age.

Leaves ovate lanceolate; apex acute; base rounded; margin finely and evenly glandular serrate; upper surface dull green with scattered hairs; under surface grayish green with a thin silvery pubescence; 3 to 4 cm. long, 1.5 to 1.7 cm. wide; venation prominent; petioles 4 to 5 mm. long; pubescent; fruit solitary as far as seen, a small roundish plum with the surface covered with scattering hairs; stalk 3 to 4 mm. long; not seen in mature condition. While an evident hybrid with distinct plumlike



characters, this variety retains more of the *Prunus texana* characters than any other hybrid noted. But one plant discovered, south of Big Sandy Creek, Llano County, Tex.

***Prunus texana* hybrid.**

Hort. var. Sumlin.

An erect, slender-branched shrub, with grayish brown bark on old wood and slender, yellowish brown pubescent twigs of young growth.

Leaves ovate elliptic, acute at apex, rounded or broadly wedge-shaped at base, serrate with glandular teeth; the upper surface dull green, with short scattered hairs; lower surface grayish green; hairy tomentose; 4 to 5 cm. long; midrib rather conspicuous; petiole short; stipules 3 to 4 mm. long, narrow, acute, glandular toothed.

Fruit a small, roundish, pubescent-coated plum, upon a stalk 4 to 10 mm. long. Not seen mature, but described as red in color and a desirable fruit, ripening somewhat later than the *Prunus texana* parent. Some of the characters in this variety suggest that the cross may have been derived from a local wild plum usually classed as *P. americana* var. *lanata* Sudworth, though perhaps an undescribed species. Trees of this form occur in the same field and, while flowering a little later, overlap *P. texana* in blooming period.

***Prunus texana* hybrid.**

Hort. var. Holmann.

An erect-growing shrub 1 to 2 meters high, of irregular branching habit, inclined to be spiny. Young growth slender, yellowish brown, with thin pubescence; older wood iron gray.

Leaves 3 to 5 cm. long, 1.5 to 2 cm. broad, ovate lanceolate, with rounded base and acute apex; margin finely glandular serrate; upper surface with scattered short hairs; lower thinly pubescent; petiole 4 to 6 mm. long.

Fruit a small oval plum with a thinly pubescent surface, borne singly or in pairs; stalk 6 to 10 mm. long; calyx sometimes persistent. Described as being of poor quality. Found in a scattering group of small thickets, indicating that it has ability to spread by root sprouts.

***Prunus texana* hybrid.**

Hort. var. Gephart.

A tree 2.5 meters high, with numerous slender semipendulous branches; young growth reddish brown, finely pubescent; older wood silvery gray or iron gray.

Leaves narrowly elliptical, approaching oblong; apex rounded or acute, finely doubly serrate with minute glandular teeth; base rounded or broadly wedge shaped; upper surface dull green, covered with scattering short hairs; lower surface ashy gray green, finely reticulated, silvery pubescent; 3 to 4 cm. long; stipules 2 to 3 mm. long, slender, acute, glandular toothed.

Fruit borne in great profusion, smooth, plumlike in appearance, oval, 2.5 cm. long, dull yellow, with slight pubescence; stalk 3 to 5 mm. long; a juicy fruit, the rather soft flesh clinging to the pilose pit much as in the original species, somewhat subacid and lacking in quality. The earliest ripening of any of the *Prunus texana* hybrids so far noted (May 13 to 18).

***Prunus texana* hybrid.**

Hort. var. Johnson.

This variety was found growing interlocked with the Gephart, but is more upright and stiff branched in habit and quite distinct. Young twigs reddish brown, slightly angled at the nodes, sparingly pubescent; older growth grayish brown or iron gray. (Pl. X, fig. 3.)

Leaves narrowly elliptical or obovate elliptical, rounded at the apex, rounded or tapering at the base; margin finely doubly glandular serrate, dull pale green set with scattered hairs above, ashy green, thinly pubescent beneath, 3 to 4.5 cm. long, 1 to 1.5 cm. or, rarely, 2 cm. broad; the midribs and slender petioles, which are 1 to 2 cm. long, are dull purplish; stipules 2 mm. long, slender, acute, glandular toothed.

Flowers not seen.

Fruit in close bunches, single or paired, 2 to 2.3 cm. long, oval, slightly compressed, covered with a fine, soft pubescence; stalk slender, 1 cm. long, pubescent, inserted in a very slight depression. Skin dull greenish yellow, tough; flesh greenish yellow, acid, flavor better than that of the Gephart, but not a fruit of high quality; stone oval, flattened, acute at apex, having a soft, short, velvety pile of the *Prunus texana* type.

***Prunus texana* hybrid.**

Hort. var. Bolen.

A compact, pendulous-branched tree, about 2.5 meters high, with finely pubescent, brown twigs of young growth.

Leaves broadly elliptical, narrowing abruptly to an acute apex; base rounded or broadly wedge shaped; margins finely glandular serrate, upper and lower surfaces with scattered silvery hairs, scarcely amounting to a pubescence, 4 to 4.5 cm. long, 2 to 2.5 cm. broad, the yellowish brown midrib passing into a slender hairy petiole, 5 to 7 mm. long.

Fruit 2.5 cm. long, 1.5 to 2 cm. broad, oval, slightly oblique, and tapering to an obtuse apex; stalk about 5 mm. long, a little stouter than in the Gephart variety. Skin dull yellow, rather tough; flesh yellow, rather thin because of the large seed; flavor very similar to that of the pure *Prunus texana* species.

***Prunus texana* hybrid.**

Hort. var. Stuart.

A small tree with trunk 1 cm. in diameter and spreading top 2.5 meters high and 3 meters broad; branches angular but smoother and more open than in *Prunus texana*; bark grayish brown. The trees show some tendency to spread from root sprouts.

Leaves ovate elliptical, rounded or broadly pointed at apex; cuneate at the base; serrate or doubly serrate, with fine glandular teeth; dull green with fine scattered hairs on the upper surface; grayish green, finely pubescent beneath; 3 to 3.5 cm. long; a conspicuous midrib passing into a short, dull red petiole; stipules minute coarsely glandular toothed.

The flowers, which appear later than the leaves, borne singly or two or three in a fascicle, are about 6 mm. in diameter, on slender hairy pedicels from 4 to 8 mm. long; calyx tube narrowly campanulate, surface sparsely covered with short hairs; lobes elliptical, with scattered glandular teeth and fringed with fine hairs; inner surface with scattered hairs; petals thin, white, broadly obovate, with a short claw; ovary velvety pubescent, but style smooth. Mature fruit oval, about 2.5 cm. long, with an acute cavity around the short stalk; dull yellow, with velvety surface and mellow, luscious, highly flavored flesh. Seed oval, turgid, with heavy velvety pile.

***Prunus texana* hybrid.**

Hort. var. Hilderbrand.

A small tree with slender, erect, rather angular branches; bark smooth, grayish. Leaves obtuse or rounded at the ends; finely, sometimes doubly, serrate, with minute glandular teeth; dull green, with scattered fine hairs on the upper surface; grayish green with hairs more numerous below; 3 to 3.5 cm. long, 0.7 to 1 cm. wide; midrib narrow, tinged with dull purple at the base; petiole short, slender, pubescent; stipules 2 mm. long, narrow acute, glandular serrate.

Flowers not seen, apparently opening with the leaves.

Fruit oval, velvety, stalk 1 cm. long, slender, nearly glabrous. (Mature fruit not seen; described as being red.)

***Prunus texana* hybrid.**

Hort. var. Whittaker.

A shrub of treelike form, 2 meters high; branches regular or somewhat angled at the nodes, long, slender, with few spines; bark smooth, iron gray or brown.

Leaves thin, narrowly elliptical, acute at both ends, doubly serrate with minute glandular teeth; dull green with minute scattered hairs above, grayish green, more abundantly hairy below; 4 to 5 cm. long, 1 cm. to 1.3 cm. broad; petiole slender, pubescent, dull purple, 0.5 to 1 cm. long; stipules lanceolate, acute, glandular serrate, about 2 mm. long.

Flowers, appearing with the leaves, small, on slender hairy peduncles about 6 mm. long (petals not seen); calyx tube narrowly elliptical, fringed with fine silvery hairs and sparsely coated with hairs on the inner surface.

Fruit borne singly or in pairs, oval, finely pubescent (not seen mature; color said to be red), the pubescent stalk 6 to 8 mm. long. •

THE NEVADA WILD ALMOND •

The wild almond (Pls. XI and XII, figs. 1 and 2), the most striking of all the dry-fruited members of the plum family occurring in the United States, was first described by Asa Gray from specimens sent him by Dr. C. L. Anderson, collected near Carson, Nev., 1863-1866, and was named in honor of Dr. Anderson.

From field notes and abundant herbarium material collected by the writer in person or supplied by Mr. E. W. Hudson, important characters heretofore unnoted are brought out and this species is redescribed as follows:

***Prunus andersonii* Gray.**

*Prunus andersonii* Gray, Proc. of Amer. Acad., v. 7, p. 337-338, 1868.

*Amgdalus andersonii* (Gray) Greene, Fl. Franc., pt. 1, p. 49, 1891.

*Emplectodadus andersonii* (Gray) Nelson and Kennedy, Muhlenbergia, v. 3, p. 139, 1908.

Illus., Schneider, C. K., Laubhk., p. 598, fig. 335, d, e.

A spiny, much-branched, interlocking shrub 1 or 2 meters high, or, rarely, more smooth, erect, and treelike, reaching 3 meters or over; bark of young branches grayish green to reddish or yellowish brown, glabrous, on older wood breaking into coarse, dark-gray scales. The leaves are convolute in the bud, broadly or narrowly spatulate, with rounded or acute apex and short petiole, finely serrulate or entire, often with a pair of small glands near the base, 1 to 4 cm. long; yellowish or grayish green, leathery, glabrous, or faintly pilose at the base; stomates present in the upper epidermis.

The flowers, appearing with the leaves, are perfect, 1.5 to 2 cm. in diameter, on slender glabrous pedicels, 1.5 cm. or less in length, solitary or fascicled; calyx tube short, campanulate, leathery, glabrous, or rarely with pedicel and calyx cup puberulous; lining nectariferous; the lobes triangular with ciliate margins, often persistent on mature fruit; petals from pale to deep-rose color, or rarely white, oval, 6 to 10 mm. long, narrowing abruptly to a short claw; stamens 20 to 30; style equal to or longer than the stamens; glabrous or only the lower one-fourth hairy; ovary pubescent.

Fruit roundish or obliquely unsymmetrical, compressed, often with a marked winglike ventral expansion, abruptly rounded to an apiculate apex; base distinctly

necked, 1 to 1.8 cm. long, dull grayish or greenish yellow with thickly pubescent surface, usually with prominent, coarse, reticulate venation as it dries; the thin flesh dry, leathery, and astringent, or, rarely, more succulent and with edible qualities, usually splitting along the ventral suture at maturity after the fashion of an almond.

Stone roundish, unsymmetrical, turgid or compressed, the narrow dorsal wing having a shallow groove; the ventral wing often much expanded; has an acute central ridge usually flanked by parallel ridges and obscure reticulate veins; surface smooth

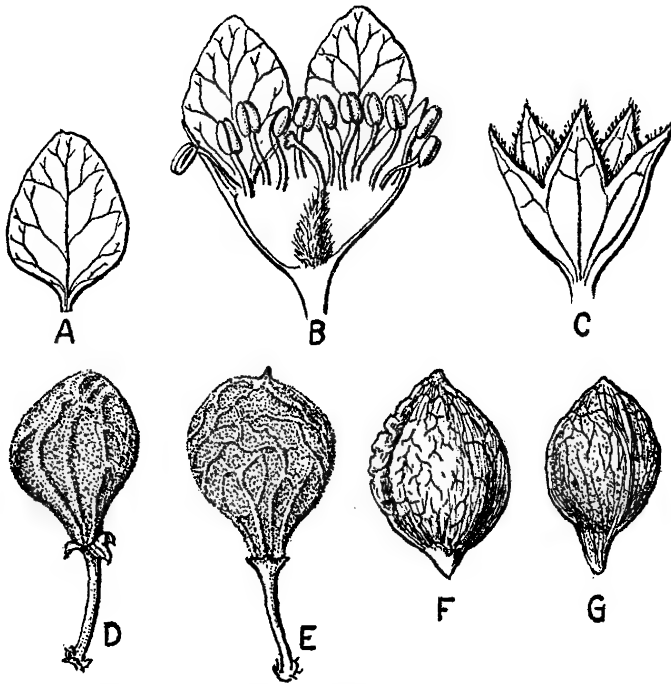


FIG. 4.—*Prunus andersonii* Gray: A, Petal,  $\times 3$ ; B, section of a flower,  $\times 3$ ; C, calyx showing ciliate margins,  $\times 3$ ; D, E, dried fruit  $1\frac{1}{2}$  times natural size; F, G, stone,  $1\frac{1}{4}$  times natural size.

or obscurely or decidedly pitted; apex rounded to an acute point, base with a more or less thin, attenuated neck; kernel small, pointed, grooved in some varieties, edible, often strongly flavored with prussic acid. (Fig. 4.)

This species is one of the most distinctive of those commonly included in the *Emplectocladus* group.

On mountain sides and dry foothills of eastern California and Nevada it is a squarrose, much-branched and spiny shrub, 1 to 2 meters in height and diminishing to 0.5 or 0.7 meter at its upper limit of growth. In more favorable situations, along the shore of Pyramid Lake and other localities where better soil and a more constant supply of water occur, it becomes a large shrub or even a small tree. Forms appear reaching over 3 meters in height, nearly free from spines, with clean, free growing branches and have the appearance of young peach or almond trees. (Pl. XII, figs. 1 and 2.) Well-marked varietal forms are found, not only

in habit of growth and branching, and color and texture of bark, but in size and color of flowers and character of fruit.<sup>1</sup>

One variety was noted with fruits of unusual size and having a fleshy development of the pericarp instead of the characteristic dry, leathery coating.

A very strongly developed taproot is a characteristic of this species as well as of several others of the group. This has been very noticeable in growing seedlings. Seeds stratified in sand and exposed to open conditions of a severe winter of Washington, D. C., made a vigorous germination early in March, sending down strong taproots, while the tops were but two or three leaves above the ground. This must be recognized as an adaptation which has enabled them to survive under peculiar local conditions. Where wild-almond thickets occur there can usually be traced at a depth of 1 or 2 meters a layer of soil or sand where more permanent moisture is afforded than prevails near the surface. After the taproot has penetrated this layer small branches spread out into it and the moisture made available enables the plant to survive drought and heat which would have caused it to perish if supported by superficial roots.

The range of occurrence of this species is shown on the map (fig. 1) and is a region of such scant rainfall that little agriculture is possible without irrigation. Taking Carson City, Nev., as a typical station,<sup>2</sup> the mean annual precipitation is slightly above 10 inches, falling as low as 5 inches in years of extreme drought. The 2 feet or more of snow forms a considerable portion of the annual moisture, the mean precipitation from April to September, inclusive, being but 2.4 inches. With summer heat occasionally reaching 100° F., and the average winter temperatures of -20° F., some idea of the hardiness and drought resistance of this species can be formed.

#### THE DESERT APRICOT

This striking apricotlike species occurs only in certain out-of-the-way places in southern California. (Pls. XII, fig. 3, XIII, and XIV, fig. 1.)

Confined chiefly to a narrow zone on the desert side of the San Bernardino and San Jacinto Mountains, the only frequented spots of its habitat are the village of Palm Springs at the foot of San Jacinto Peak on the south and the almost deserted hamlet of Banner at the foot of the mountains and just above the border of the desert below Julian in San Diego County.

In the Gray Herbarium the type specimen sheet has mounted upon it a specimen bearing the label "*Prunus subcordata*, Bth.," and in print,

<sup>1</sup>Mr. E. W. Hudson, of the Office of Crop Physiology and Breeding Investigations, while doing cooperative work at Wadsworth Agency, made numerous collections of this species in 1910 and noted that the flowers ranged in color from pale pink to a deep-rose color, and also varied greatly in size.

<sup>2</sup>Henry, A. J. Climatology of the United States. U. S. Dept. Agr. Weather Bureau, Bulletin Q, p. 920, 1906.

"Flora of Southern California, &c. Coll. by C. C. Parry and J. G. Lemmon, 1876." In the upper right-hand corner of the same sheet is a specimen of very different appearance bearing the label, "Frémont's Expedition to California, 1845-7. 370—1846," and in pencil "New" (in the hand of Dr. Asa Gray). At the bottom of this sheet is the penciled label "*P. Fremonti* Watson, n. sp."

The specimen first cited and referred to as collected by Cleveland in Oriflamme Canyon bears the label "*P. subcordata*, var. *erigyna*," but it has also beneath a subsequent label the penciled inscription, "*P. Fremonti* Watson, n. sp."

It has been noted by Mr. W. F. Wight, of the Bureau of Plant Industry, in a memorandum placed upon the specimen in 1910, that the Frémont specimen is *Prunus subcordata*, a determination supported by the glabrous pistils and the leaf characters.

Dr. Watson clearly had before him three specimens upon which he based his description of the new species and to which he attached the name. While he cites the Frémont specimen last we may readily presume that it was because of its lacking a definite locality label, which the first and second citations possessed. Having incorrectly included it in the type material, however, and having given the name "*Fremonti*" to the species, this specimen, according to the American Code of Botanical Nomenclature (section 4, canon 14, a), becomes the type specimen. *Prunus fremonti* Watson, then becomes a synonym of *P. subcordata* Benth., leaving the species bordering the Colorado Desert unnamed. The name *Prunus erigyna* is accordingly proposed for this species.

These two species seem to have been subject to much confusion by the earlier collectors.

Dr. Torrey in the Botany of the Mexican Boundary Survey<sup>1</sup> refers specimens collected by the expedition at San Felipe to "*Prunus subcordata*, Benth., Pl. Hartw.," yet his description tallies well with *P. erigyna*, and the San Felipe locality renders it probable that he had this species before him.

The specimen collected by Frémont is undoubtedly *Prunus subcordata* Benth., the type of which was collected by Hartweg somewhere about the upper waters of the American River in the latter part of April, 1846.<sup>2</sup>

By an interesting coincidence Col. Frémont in his Memoirs, p. 476, mentions camping March 26, 1846, at the ranch of the same Mr. Cordua where Hartweg made his headquarters in the Sacramento country. The month of April Frémont spent in the region tributary to the Sacramento River, now included in Butte, Tehama, and Shasta Counties,

<sup>1</sup> Torrey, John. Botany of the boundary. Emory, W. H. Report of the United States and Mexican Boundary Survey . . . v. 2, Washington, 1859, p. 63.

<sup>2</sup> Hartweg, T. Journal of a mission to California in search of plants. Jour. Roy. hort. soc. [London], v. 3, p. 221, 1848.

and the date of Hartweg's collection, made at a considerable altitude in the foothills, suggests the probability that the Frémont specimen was secured in the upper waters of one of the many mountain tributaries which he visited.

From abundant material collected near Palm Springs and in the Banner Canyon of San Diego County and from field notes covering several seasons' observations the following detailed description of this new species has been drawn.

***Prunus eriogyna*, n. sp. (Fig. 5.)**

*Prunus fremonti*<sup>1</sup> S. Watson, in California, Geological Survey, Botany, v. 2, Cambridge (Mass.), p. 442-443, 1880.

*Amygdalus fremonti* (S. Watson) Abrams, in Bull. N. Y. Bot. Gard., v. 6, no. 21, p. 385, Sept., 1910. Illus., Schneider, C. K., Laubhik., Lig. 5, p. 598, fig. 335, u, v.

A spiny, intricately branched and angled shrub reaching 4 meters in height. Twigs of young growth glabrous, bright reddish brown, becoming silvery gray or brown with age. Bark on old stems black, breaking into thin plates or scales.

Leaves variable, lanceolate, ovate or orbicular, or sometimes broader than long, rounded or cordate at the base, narrowing abruptly to a short acute apex or often rounded or obtuse; glandular denticulate, usually with one or more larger glands near the base or rarely on the petiole; both surfaces pale grayish green, shining above, firm, sometimes leathery; midrib and veins prominent on under surface; stomates in both upper and lower surfaces; 1.5 to 3 cm. long, 1.5 to 2.5 cm. or more broad; petiole 6 to 8 mm. long; stipules minute, narrowly acuminate, glandular denticulate.

The perfect flowers, borne in small umbels and having a faint, agreeable odor are produced in great profusion, appearing from January to March, according to rainfall, when the leaves are partially developed. In-bud they are white, salmon, or rose pink. Expanded they are usually 6 to 8 mm. in diameter, reaching 18 mm. in some forms, on slender pedicels 6 to 12 mm. long; calyx tube short, campanulate; outer surface glabrous or thinly pubescent; inner covered with a salmon or rose colored pigment; lobes oval, half as long as the petals, finely pubescent on inner surface, glandular ciliate, often hanging loosely in a dried condition around the pedicels of the mature fruits; petals white, pink, or rose, 3 to 6 mm. long, oval, incurved at apex, base rounding to a stout claw; stamens about 24 to 30, many imperfect; ovary and lower portion of the style finely pubescent; stigma but little expanded.

The fruit, which ripens in May, is in appearance a small apricot, 1 to 2 cm. long, subglobose, ovoid or oblong ovoid, sometimes oblique, slightly or decidedly compressed; apex mucronate; skin puberulent, dull yellow or greenish yellow, often with a dull-rose flush, with a well-marked ventral suture along which the thin astringent flesh opens in ripening, sometimes allowing the stone to drop, while the desiccated flesh remains attached to the peduncle; stone smooth or slightly roughened, usually flattened or somewhat turgid, obtuse at both ends with a well-marked dorsal furrow and a thick ventral expansion along the middle of which is a low, acute ridge separated by smooth, narrow furrows from two obtuse parallel ridges; often one or more pairs of obscure veins extend from the base and branch along either side; stony walls thick,

<sup>1</sup>"*P. Fremonti*. A spiny glabrous densely branched shrub or small scraggy tree (15 feet high) with short branchlets: leaves small (4 to 8 lines long), thin, ovate or roundish, on short slender petioles, denticulate: flowers appearing with the leaves, solitary or somewhat fascicled, 5 or 6 lines broad, on pedicels 2 or 3 lines long: calyx lobes ciliate: ovary densely pubescent; style elongated: stone oblong, turgid, rounded on one side and with a broad ridge upon the other, 5 lines long.

"Coast Ranges of Southern California, Oriflamme Cañon, San Diego County (*D. Cleveland*); San Bernardino Mountains, *Parry & Lemmon*, n. 108, 1876. Also collected by *Fremont* in 1846, locality uncertain. Flowering in March; fruit probably with little pulp."

kernel small, strongly flavored with prussic acid. Type specimen in United States National Herbarium, C. P. B. No. 1155. Merotypes cut from the tree that yielded the type specimen have been sent to a number of other herbaria.

The type locality of *Prunus eriogyna* is along the watercourse in the boulder talus at the mouth of Tahquitz Canyon at the southern base of the San Jacinto Mountain, near Palm Springs, Riverside County, Cal. It is also found on dry talus slopes in Andr  as, Murray, and Palm Canyons, along the trail to Van Deventer Flats below Santa Rosa Peak, and up the rocky slopes of the San Jacinto Mountains to an altitude of over 2,000 feet, growing in barren soil and crevices of rocks, being apparently extremely xerophytic. Its range is from the southern slopes of the San Bernardino Mountains southward along the desert slopes of the San Jacinto Mountains to San Diego County, and into Lower California.

The plumlike appearance of the wood, especially of the younger growth, and perhaps a sprinkling of roundish or oblong green pubescent-coated fruits, would excite an inquiry that would bring out the names "desert almond" or "wild apricot."

#### ADAPTATION TO DESERT CONDITIONS

The adaptations of *Prunus eriogyna* to the peculiar conditions which prevail on the desert slopes of mountains are worth noting. The rainfall, slight as it is, really governs plant activities, and vegetation becomes most nearly dormant during the summer months. The rains consist of rare torrential downpours in August and light rains from October to May, but are nearly confined to the period from December to March, the entire volume ranging from less than an inch to 9 inches and a fraction annually. With warm winter days and the temperature at night falling but little

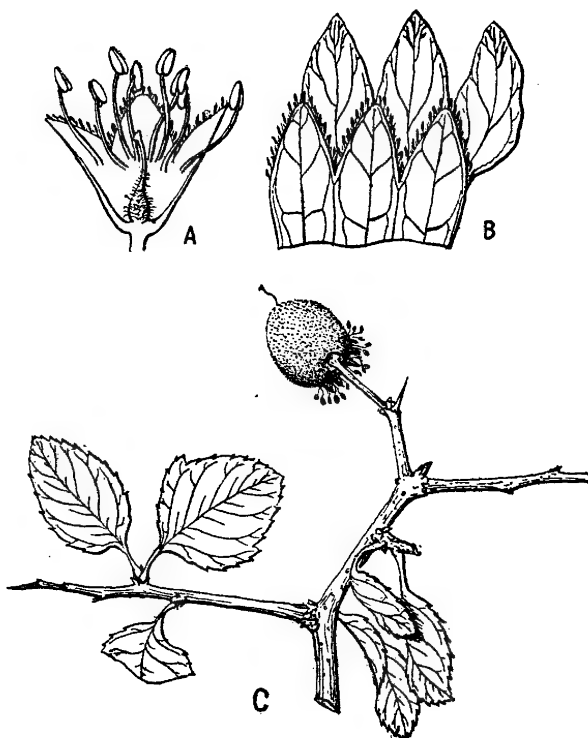


FIG. 5.—*Prunus eriogyna*, n. sp.: A, Section of calyx,  $\times 3$ ; B, detail of portion of calyx with petals, from outside, showing glandular ciliation of lobes,  $\times 3$ ; C, twig showing angular habit of branching, leaves and fruit attached,  $\frac{3}{4}$  natural size.



below freezing the vegetative activity in many species of plants that have become dormant during summer drought may be resumed at any time when a sufficient supply of water is afforded.

In the case of *Prunus eriogyna* a copious November rain may start the favorably located bushes into activity, so that a small percentage of the many flower buds will open in January. Cool nights and light frosts may destroy a portion of these buds, but some will set fruit, indicating a fair degree of hardiness for this species. At the time of the main flowering in March there may be a few scattering, nearly mature fruits, which escape the numerous plum curculios and furnish a small supply of seeds for germination should the rainfall be inadequate to mature the main crop of fruit.

In seed germination this species differs strikingly from ordinary apricots or plums. Germination is rapid, the plants appearing above the ground in from 8 to 10 days. As an example, in a pot of seeds sown in sandy soil in a greenhouse on July 31 a number of plants were above the soil on August 6. One with the plumule 1 cm. long had already sent down a taproot of 9 cm. In desert conditions with fruit ripened in May germination is necessarily deferred till the autumn or winter rains set in, when the quick germination habit is essential to its success. Getting its roots down to a zone of permanent moisture, however slight, is the necessary thing if the seedling is to survive the dry, hot summer that follows. A sufficient leaf expansion to afford the needed root growth is all that is necessary and more would only hasten transpiration and waste the limited supply of moisture.

That even the best forms of *Prunus eriogyna* are far from having the quality of cultivated apricots is evident from the appearance of the plants, but that this desert species of the Pacific slope has very close affinities with the true apricot of the Orient can not be doubted. The apricot relationship of *P. andersonii*, with which is placed *P. eriogyna*, is not so evident, yet its convolute leaves, fascicled flowers, and slender-stalked fruit with a slight tendency to be fleshy will ally it to the *Prunus dasycarpa* type of the apricot more nearly than to the almond.

#### THE CALIFORNIA DESERT ALMOND

The desert almond, also called the "wild peach" and "wild almond," occupies a range much farther south and east than that of the Nevada wild almond, *Prunus andersonii*. It overlaps the southern range of *P. andersonii* in Nevada and eastern California and that of *P. eriogyna* in southern California. It has been collected near the coast in San Luis Obispo and Santa Barbara Counties and as far east as southwestern Utah and northwestern Arizona. Its greatest abundance as far as studied is along the foothills bordering the Mohave Desert in the neighborhoods of Hesperia and Neenach at altitudes of 3,000 to 3,500 feet. The soils

it favors seem to be from decomposed granite or mica schist. In washes where the sands and silts from these rocks are deep an enormous root development is made, the plants forming dense thickets of many sprouts, reaching 7 or 8 feet in height. On granitic slopes above the washes the plants occasionally grow with a single stem and a miniature tree-like form (Pl. XIV, fig. 2). The following description of this species is the result of examination of many plants in the field and the study of abundant herbarium material.

***Prunus fasciculata* Gray. (Fig. 6.)**

*Emplectocladus fasciculatus* Torr., Pl. Frémont, p. 10-11, pl. 5, 1853.<sup>1</sup>

*Prunus fasciculata* Gray, Proc. of Amer. Acad., v. 10, p. 70, 1875.

*Amygdalus fasciculata* Greene, Fl. Franc., pt. 1, p. 49, 1891.

Illus., Schneider, C. K., Laubhk., Lfg. 5, p. 598, fig. 335, f, g, h; Torr., loc. cit.

A much-branched, scarcely thorny shrub, with many small branched stems from a common crown or rarely with a single stem and short stiff branches, usually 1 or 2, rarely 3 meters high, with stems 6 to 10 cm. in diameter at the base.

The bark on young twigs is usually puberulous or pubescent, at first pale green, darkening to reddish or silvery brown, with conspicuous lenticels; dark gray brown or nearly black on older wood.

The leaves, conduplicate in veneration,<sup>2</sup> are borne singly on young wood of free growth, but are fascicled on short budlike suppressed branchlets on older growth. They are narrowly linear spatulate with a mucronate apex and cuneate base; margin entire or with a few fine serrations; blade thin, pale green, puberulous above and

below; 1 to 4 cm. long, 3 to 7 mm. broad; petiole short or wanting; stipules caducous, slender, attenuate, minutely glandular.

The flowers, dioecious by abortion of stamens or pistils, are minute, solitary or paired, sessile or very short stalked. In the staminate form the calyx tube, about 3 mm. long, is obconic campanulate, with blunt triangular teeth; glabrous or faintly

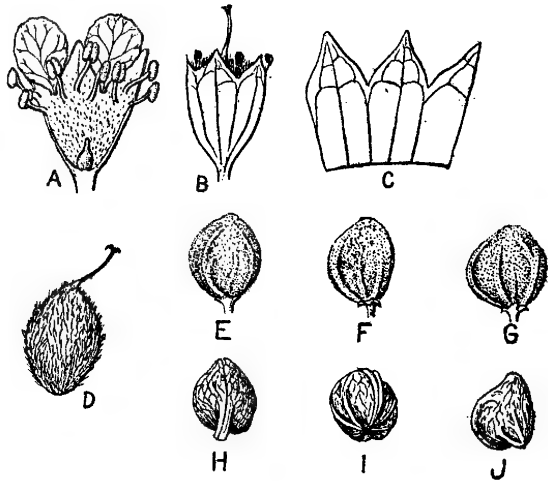


FIG. 6.—*Prunus fasciculata* Gray: A, Section of staminate flower, showing abortive ovary and minute hairs on interior of calyx,  $\times 3$ ; B, calyx cup, pistillate form, showing abortive stamens,  $\times 3$ ; C, detail of calyx lobe,  $\times 5$ ; D, fecundated ovary,  $\times 3$ ; E, F, G, fruits, three forms, natural size; H, I, J, seed, dorsal, ventral and side views, natural size.

<sup>1</sup> From incomplete material collected by Gen. Frémont this species was made the basis of a new genus by Dr. Torrey in 1853, the Latin description of which is rendered in English as follows:

*Emplectocladus* n. gen.—Calyx obconical campanulate; tube not at all contracted at the naked throat; limb divided into five equal parts, persistent. Petals 5, erect-spreading. Stamens 10 to 13, biserial, pistils 1 to 2 (generally solitary), unilocular; ovules two, collateral, pendulous. Style very short, thick, slightly oblique, stigma capitate. Fruit ———.

California shrub, very much branched; branches rigid, spreading, subspinescent; leaves minute, spatulate, from subglobose buds, almost fascicular; stipules minute, deciduous; flowers subsolitary, sessile terminal, small.

<sup>2</sup> Only the most careful inspection of very young leaves as they emerge from the bud will discover that they are conduplicate. The adhering margins of the linear-spatulate leaves hold them in a tubular form as they expand, giving them a rolled appearance which is accented by a slight twist.

puberulous without and minutely hairy on the inner surface. Ten or twelve stamens on short filaments are arranged in two series. The petals, 2 mm. long, are white, broadly obovate cuneate, with erose margins and without claw.

In the pistillate form the calyx tube is rather more campanulate. There are minute abortive stamens and the pubescent ovary is surmounted by a smooth style, 2 to 3 mm. long. The mature fruit, borne on a very short peduncle, is coarsely pubescent, irregularly globose, 1 to 1.3 cm. long, having a distinct ventral ridge with a shallow furrow through the center and two or three pairs of small concentric ridges arising from the base and disappearing toward the rounded apiculate apex.

The thin, dry pericarp does not split as in *Prunus andersonii* and *P. eriogyna*. The thin-walled stone is smooth surfaced excepting minute sharp ridges corresponding to those of the outer surfaces. Kernel scarcely edible because of the strong prussic-acid flavor.

Mr. F. V. Coville seems to have been the first to notice that the flowers of this species were otherwise than perfect. His description contains the following paragraph:

The flowers are polygamo-dioecious, a fact which explains Dr. Gray's difficulty<sup>1</sup> in identifying Torrey's plants with others subsequently collected. In the prevailing male flowers the petals in our specimens are elliptical lanceolate, appressed strigose on the back, 3 to 3.5 mm. long; the filaments 2 mm. and the anthers 1 to 1.2 mm. in length, while the style is 1 to 2 mm. long, and the pistil sterile. In the fertile flowers the petals are ovate, glabrous on the back, 2 to 3 mm. long, the filaments 0.6 to 0.8 mm., the anthers 0.4 mm., and devoid of pollen, and the style about 2 mm. long. The sterile flower is the one figured by Torrey (loc. cit., pl. v). The form and length of the petals probably vary considerably.<sup>2</sup>

Schneider<sup>3</sup> recognizes this and the two following species as "subdioecisch" (subdioecious).

#### THE TEXAS ALMOND

The Texas almond, first collected by Lindheimer south of New Braunsfels, Comal County, Tex., "not far from Cebolo Cr.," occurs in the northwest suburbs of San Antonio and occupies an imperfectly known region southwestward to the Rio Grande and beyond<sup>4</sup> apparently restricted to the limestone soil of the Cretaceous formation. (Fig. 2.)

The region of the lower Pecos near the Rio Grande is one of deep deposits of soft cretaceous limestone rock, deeply eroded and very broken. The soil over the hills is often very thin or the bare rock is wholly exposed. In the broader washes some soil is beginning to collect in the form of miniature bottom lands, occasionally overflowed by the run-off from heavy rains. Along these washes there is sometimes a fringe of scrubby growth of hackberry, oak, the western black walnut (*Juglans rupestris*), the "chapote," or Mexican persimmon (*Diospyros texana*), and similar arid land forms. It is in these situations that the Texas almond is found

<sup>1</sup> Proc. Amer. Acad. x., p. 70 (1874).

<sup>2</sup> Coville, F. V. Botany of the Death Valley expedition. Contrib. Nat. Herbarium. v. 4, p. 91, 1893.

<sup>3</sup> Schneider, C. K. Illustriertes Handbuch der Laubholzkunde, Bd. 1, Lfg. 5, Jena, 1906, p. 598.

<sup>4</sup> "... Gravelly places and ravines between Devil's River and the Rio Grande; also in Chihuahua; Parry. Bigelow." Torrey, John. Botany of the boundary. Emory, W. H. Report of the United States and Mexican Boundary Survey . . . v. 2, Washington, 1859, p. 63.

rather than in strictly upland conditions, though in a few instances it was found on high ground, where it benefited by no addition to the rainfall by means of run-offs.

The Texas almond is a shrub scarcely 6 meters high in its northern range. Where it was studied by the writer in Valverde Co., Tex., along the limestone washes, it frequently forms thickets from 1 to 1.6 meters in height, with stems 2 to 3 cm. in diameter.

The dioecious habit of this plant is one of its most marked characteristics, when one has the opportunity of examining the plants in large numbers in its most favorable conditions.

The bushes bearing the staminate flowers are much more numerous than the fruiting ones and the flowers more numerous and crowded, so that in the field it is generally possible to distinguish the types from a distance. The examination of a large number of plants in flower in Valverde County failed to show a single case in which the flowers could be called polygamo-dioecious. In no case were hermaphrodite and unisexual flowers found on the same plant. Not a pistillate flower was found with fertile stamens nor a staminate flower that did not have the pistil abortive and much reduced in size.

During seasons of drought and scarcity of forage these bushes are browsed by stock on these ranges. In the suburbs of San Antonio, where the grazing of cows has been heavy on vacant lots, these bushes were found cropped back to a very small size and nearly all affected with crown-gall.

Field study of two seasons of this species in flower and fruit has furnished the material for the following revised description (Pl. XV.):

***Prunus minutiflora* Engelm. (Fig. 7.)**

*Prunus minutiflora* Engelm., in Gray, A., Pl. Lindheim., pt. 2, Boston Soc. Nat. Hist., v. 6, p. 185, 1850.  
*Cerasus minutiflora* (Engelm.) Gray, in Pl. Wright, pt. 1, p. 68, 1852.  
*Amygdalus minutiflora* (Engelm.) W. F. Wight, in Dudley Mem. Vol., p. 130, 1913.  
 Illus., Schneider, C. K., Laubhk., Lfg. 5, p. 598, fig. 335, m, n, o, p.

An erect but much-branched, angled and spiny shrub, from 0.5 to 1.6 meters high, stems 1 cm. to 3 cm. in diameter, forming considerable thickets along limestone slopes and washes in the cretaceous section of central and western Texas.

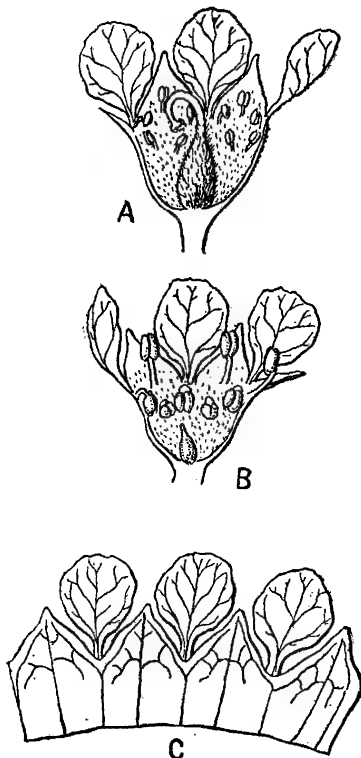


FIG. 7.—*Prunus minutiflora* Engelm.: A, Section of flower of pistillate form, showing well-developed pistil and abortive stamens,  $\times 4$ ; B, section of flower, staminate form, showing well-developed stamens and abortive pistil,  $\times 4$ ; C, detail of calyx lobes and petals.  $\times 4$ .

Twigs of young growth often puberulous, reddish brown or silvery gray; older wood with silvery gray or iron-gray bark.

The leaves, which are conduplicate in the bud, borne singly on young growth but fascicled on short spurs on older wood, are spatulate or narrowly elliptical; apex rounded, retuse or mucronate; base cuneate, entire or with one to several minute teeth on either margin, and rarely one or two near the base, glandular tipped, firm and leathery, pale bluish green, glabrous or faintly puberulous at the base, 1 to 3 cm. long, 0.5 to 1 cm. wide; petiole short, slender; stipules 2 mm. long, acuminate, ciliate margined.

The minute flowers, borne singly or paired, on short peduncles, are usually crowded on short, budlike fruiting spurs. They appear with the leaves in February or March and are minute and dioecious by the abortion of the stamens in the fruiting form and of the pistils in the opposite form. In both types the inner surface of the calyx is finely hairy. In the pistillate type the calyx tube is obconic, glabrous; lobes triangular, acute; peduncle 3 mm. long, puberulous; ovary and lower portion of the style finely pubescent. There are usually 15 or more abortive stamens. Petals white, about 2 mm. long, obovate cuneate, with sinuous or crose margins and short, stout claws.

In the staminate flowers the tube is slightly broader, the stamens 10 to 15 or rarely 16 to 20 on short filaments, usually with a stamen opposite each petal, one or two against each calyx tooth, and an irregular number disposed on the upper surface of the tube. The pistil is abortive and much reduced.

Fruit globose, apiculate and with shallow ventral furrow, pubescent, 1 to 1.5 cm. long, the thin, dry sarcocarp scarcely dehiscent; the stone smooth with but a slight furrow on ventral surface.

#### THE MEXICAN ALMOND

The Mexican almond was the first of this group to be described, but to-day is the least known of all of them. Found in the high mountain regions of Mexico, it has been little collected and it is not known that it has as yet been brought into cultivation.

Judged by the pubescent thin-fleshed fruit with its smooth, oval stone its relationship would be considered near to the Texas almond (*Prunus minutiflora*) which crosses the border into Chihuahua, but its more slender and less spinose twigs and especially the serrate, finely pubescent leaves indicate that it is a quite distinct species. In 1823 Humboldt and Bonpland found it growing in arid hills between Pachuca and Moran (Estado de Hidalgo) at an altitude of 7,800 feet and describe it as a shrub 3 feet high with sparse, reflexed, divergent, glabrous branches and subangular pubescent twigs.

Parry and Palmer collected this shrub in the region of San Luis Potosi at an altitude of 6,000 to 8,000 feet, which would agree well with the altitude at which the original specimens were collected by Humboldt and Bonpland.

The majority of specimens in American herbariums have been collected by Mr. C. A. Purpus, of the University of California, to whom the writer is indebted for the most recent information on the occurrence and habits of this species.

The following description of this species is made from material in the United States National Herbarium, specimens contained in the herbarium

of the University of California, and material collected by Mr. Purpus for the writer:

***Prunus microphylla* Hemsley. (Fig. 8.)**

*Amygdalus microphylla*, H., B., and K., Nov. Gen. et Sp. Pl., v. 6, p. 243, pl. 564, 1823.<sup>1</sup>

*Prunus microphylla* (H., B., and K.) Hemsley, Biol. Centr. Amer. Bot. v. 1, p. 118, 1879.

Illus., Schneider, C. K., Laubhk., Lfg. 5, p. 598, fig. 335, q, r, s, t; H., B., and K., loc. cit.

A low branching shrub with slender twigs destitute of thorns; puberulous on new growth, sometimes also on wood of second year, bark greenish or reddish brown, turning to silvery or dark gray on older wood. Leaves narrowly elliptical or on fresh shoots broadly lanceolate; base slightly produced or cuneate; margin crenately serrate with blunt glandular or callus tipped teeth; dull green, faintly puberulous above; grayish green with scattered short hairs on the lower surface; nearly glabrous on old growth; 1.5 to 2 or 3 cm. long; petiole short, puberulous; stipules 2 to 3 mm. long; slender attenuate, russet, hairy with glandular teeth; stomates not present in upper surface of the blade.

The flowers, appearing in April or May before or with the leaves, are solitary, minute, and dioecious by the abortion of the stamens or pistils.

Staminate flowers sessile, with glabrous campanulate calyx tube 2 to 3 mm. long; lobes short, triangular, with expanded base and glandular ciliate margins; tube minutely hairy within; petals white, broadly obovate, entire or with notched or erose margins. Claw short or wanting. Stamens on filaments 1 to 2 mm. long are 10 to 15 or 18 in two or three circles, one circle opposite the petals, one opposite the calyx lobes near the throat, and a more or less complete circle below these. (One flower had 15 stamens and the three circles complete.) The pistil is minute, glabrous, and abortive.

In the pistillate form the stamens, with very short filaments, are abortive; the pistil, 4 to 5 mm. long, has the ovary and lower portion of the style pubescent; stigma expanded.

The mature fruit is 1 to 1.5 cm. long, oval with about equally rounded ends, apiculate by persistence of the style, but little compressed, densely rusty pubescent; sarcocarp

<sup>1</sup> Their description is translated as follows:

*Amygdalus microphylla*. Tab. DLXIV.

*Amygdalus oblong*, acute, mucronate, crenate-serrulate with glabrous leaves.

Grows on arid hills, between Pachuca and Moran, alt. 1,300 hex. (7,800 ft.). (Mexico) Shrub. Flowers in May.

Shrub 3 feet high, very much branched; branches spreading divergent, reflexed, rounded, smooth, glabrous, blackish; twigs subangular, pubescent. Leaves sparse, petiolate, densely fasciculate on shortened branches, oblong, acute and mucronate, somewhat acute at the base, crenate-serrulate, the teeth with glandular midrib, reticulate-veined, prominent below, membranaceous, glabrous, with scattered, very minute scurfy dots above, 5 to 6 lines long, 2 to 2½ lines wide. Petioles 1 line long, canaliculate, puberulous. Stipules linear-subulate, serrulate-glandular below, pubescent, twice as long as the petiole. Flowers axillary, solitary, with very short peduncles, scarcely as large as the flower of *Amygdalus incana*; peduncle scarcely half a line long, thick, glabrous, subtended by several imbricate, ovate, purplish, glabrous bracts. Calyx (figs. 1 to 3) subtrubinate-campanulate, limb 5-parted, reddish, glabrous, later split around above the base and deciduous, with ovate laciniae, denticulate-glandular at the margin, 3-veined, equal, reflexed. Petals (fig. 5) five, inserted in the throat of the calyx, alternating with the laciniae of the latter and twice as long, unguiculate, obovate, entire (2-parted fide Bonpl.), white, glabrous (this I saw formerly in specimens no longer at hand), fallen from the specimens at hand. Stamens (fig. 4) about 14, slightly shorter than the laciniae of the calyx; of these 4 inserted in a tube towards the middle; 10 around in a border (five opposite the laciniae of the calyx, five opposite the petals). Filaments subulate, glabrous. Anthers subrotund, affixed dorsally, exposed (figs. 6 to 7), deeply trisulcate in front, bilocular, longitudinally dehiscent on the inside. Ovary (figs. 8 and 9) free, sessile, oblique ovate, somewhat compressed, shorter than the calyx tube, sericeous, unilocular (fig. 10); ovules (fig. 11) two, ovate, side by side, suspended below the apex, pendulous. Style terminal, filiform, exserted, glabrous. Stigma (fig. 12) dilated, peltate. Fruit (not seen) globular, monospermous (fide Bonpl.).

Varies in a 6-parted calyx.

thin and dry, probably slightly fleshy when nearly ripe, splitting tardily along the ventral suture. Three or four pairs of shallow concentric furrows sometimes radiate from the base. Stone rounded oval with apiculate apex, smooth, with a slight ventral ridge and a faint dorsal furrow.

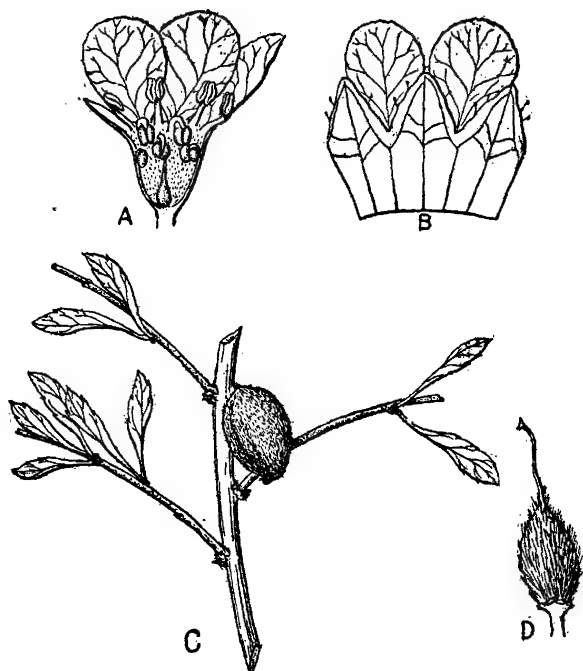


FIG. 8.—*Prunus microphylla* Hems.: A, Section of staminate flower, showing well-developed stamens and abortive pistil,  $\times 3$ ; B, detail of calyx from outside,  $\times 3$ ; C, twigs showing leaves and fruit, from herbarium specimen, natural size; D, fecundated ovary,  $\times 3$ .

*Prunus microphylla* is intermediate between *P. fasciculata* and *P. minutiflora*, but differs from both in the glandular leaf serrations. The absence of stomates in the upper surface is a noticeable difference from *P. fasciculata* and would ally this species most closely with *P. minutiflora*.

#### HAVARD'S ALMOND

*Prunus havardii* W. F. Wight, n. comb.<sup>1</sup> (Pl. XVI.)

This species, the least known of the group, was recently described by Mr. William Franklin Wight, Bureau of Plant Industry, as from specimen No. 138851, United States

National Herbarium, collected by Dr. V. Havard, United States Army, in July, 1883, at Bone Springs near the Chisas Mountains. This locality is

<sup>1</sup> "*Amygdalus havardii* W. F. Wight, sp. nov. Leaves obovate to oblong-obovate or sometimes fan-shaped on young growth, 7 to 20 mm. long, 3 to 10 mm. broad, glabrous or sometimes finely pubescent on both surfaces, usually somewhat pale below and under a lens rather prominently reticulate veined, the margin conspicuously dentate toward the apex, very rarely toothed below the middle, the teeth usually acute and apparently glandless. Flowers appearing with the leaves and sessile; calyx slightly pubescent, the tube about 2.5 mm. long, the lobes scarcely more than 1 mm. long, entire and obtuse; petals not seen. Fruit sessile, nearly globular, the pubescent exocarp dehiscent along one edge, when dry about 9 mm. long, 7 mm. broad, and 7.5 mm. thick; stone about 8 mm. long, 6.5 mm. broad, and 7 mm. thick, rounded at the base and slightly pointed toward the apex, the surface smooth except for indistinct grooves near the ventral edge.

A shrub with rather rigid branches, stout spinescent branchlets, and light gray bark. The type specimen in the United States National Herbarium was collected in fruit by V. Havard in July, 1883, in western Texas, east of the Chisas Mountains, near Bone Springs. It was also collected by C. C. Parry, J. M. Bigelow, Charles Wright, and A. Schott on the Mexican Boundary Survey under the direction of Major W. H. Emery, this specimen being labeled 'chiefly in the valley of the Rio Grande, below Doñana.' The species is most closely related to *Amygdalus microphylla* H. B. & K. of Mexico, but is easily distinguished by its broader, more obovate leaves as well as by their reticulate venation and eglandular margins." Wight, W. F. North American species of the genus *Amygdalus*. Leland Stanford Jr. Univ., Dudley Memorial Volume, p. 133, 1913.

The spelling of the specific name *havardii* is a typographical error, as the type specimen was collected by Dr. V. Havard.

in the southern part of Brewster County, Tex., at about the southern extremity of the bow of the Big Bend of the Rio Grande.

The description cites also one specimen from the Mexican Boundary Survey Collections, No. 338. As both specimens show only matured fruit, it is difficult to place this species with reference to *Prunus minutiflora* and *P. microphylla*, to which it appears to be nearly related, both in the character of the fruit and in the absence of stomates in the upper epidermis of the leaves. In its abruptly angled and thorny branchlets and nearly eglandular leaves (Pl. XVI) it would seem to be most nearly related to *P. minutiflora*. Whether it will agree with the above species in the dioecious character of the flowers, small number of stamens partly placed on the face of the calyx cup and in the finely hairy inner surface of the cup can only be determined from complete material. It is provisionally placed in the subgenus *Emplectocladus* of *Prunus*.



## DESCRIPTION OF PLATES

- PLATE IX. Fig. 1.—*Prunus texana*: Better quality of fruit. Natural size.  
 Fig. 2.—*Prunus texana*: Fruiting bush, 2 meters in diameter.  
 Fig. 3.—*Prunus texana*: Seeds; three scraped clean of pile. Natural size.
- X. Fig. 1.—*Prunus texana* hybrid, hort. var. *Stuart*: Fruit and leaves. Natural size.  
 Fig. 2.—*Prunus texana* hybrid, hort. var. *Stuart*: Tree in first leaf.  
 Fig. 3.—*Prunus texana* hybrid, hort. var. *Johnson*: Fruiting branch. Natural size.
- XI. Fig. 1.—*Prunus andersonii*: Plant, showing taproot.  
 Fig. 2.—*Prunus andersonii*: Flowering branch. Photographed by Vincent Fulkerson.  
 Fig. 3.—*Prunus andersonii*: Types of seeds. Natural size.
- XII. Fig. 1.—*Prunus andersonii*: Tangled thickets, the more common form.  
 Fig. 2.—*Prunus andersonii*: Treelike specimen, 3 meters high.  
 Fig. 3.—*Prunus eriogyna*, n. sp.: Erect, large-leaved form of plant.
- XIII. Fig. 1.—*Prunus eriogyna*, n. sp.: Common form of plant.  
 Fig. 2.—*Prunus eriogyna*, n. sp.: Variable fruits and seeds.  
 Fig. 3.—*Prunus eriogyna*, n. sp.: Fruiting branch. Natural size.
- XIV. Fig. 1.—*Prunus eriogyna*, n. sp.: Seedlings.  
 Fig. 2.—*Prunus fasciculata*: Growth in flood-swept wash.
- XV. *Prunus minutiflora*: Fruiting branch. Natural size. Photographed by S. H. Hastings.
- XVI. *Prunus havardii*: Fruiting branch of the type specimen.

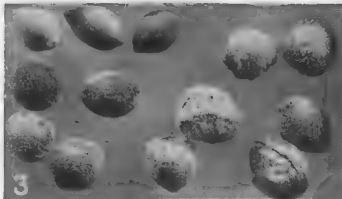
(178)

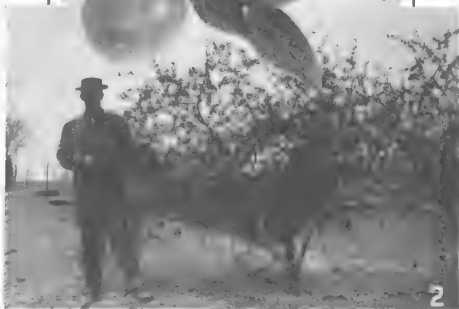
---

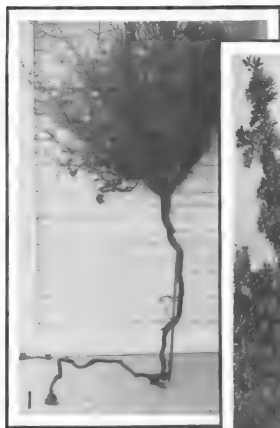
ADDITIONAL COPIES of this publication may be procured from the SUPERINTENDENT OF DOCUMENTS, Government Printing Office, Washington, D. C., at 25 cents per copy  
 Subscription, per year, 12 numbers - - \$2.50

---

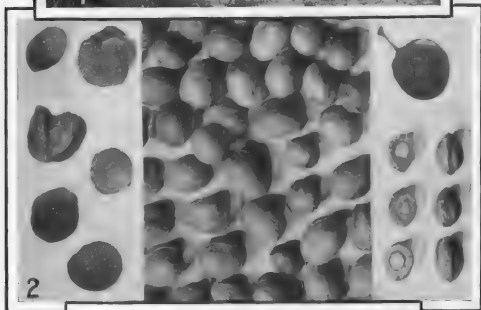
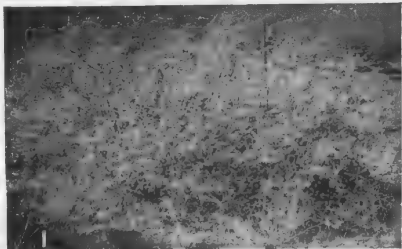


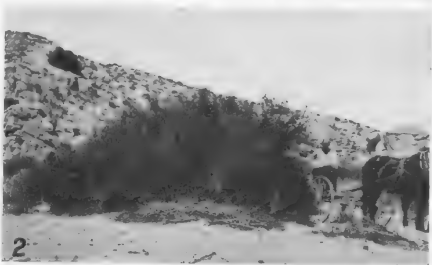


















# JOURNAL OF AGRICULTURAL RESEARCH

## DEPARTMENT OF AGRICULTURE

VOL. I

WASHINGTON, D. C., DECEMBER 10, 1913

No. 3

### SELECTIVE ADSORPTION BY SOILS

By E. G. PARKER,

*Scientist, Soil Laboratory Investigations, Bureau of Soils*

From the standpoint of soil chemistry the absorption of material from the air and the soil solution by the soil is of first importance. The absorptive power of a soil enables it to retain the soluble salts necessary to plant life in spite of the leaching effect of rains and the movement of the soil solution toward the surface of the soil in dry weather, and thus to store up soluble material, either natural or applied in the form of a so-called fertilizer, for the future needs of crops.

The absorptive properties of soils have been under investigation in the Soil Laboratory for several years under the direction of Dr. Frank K. Cameron, and several publications<sup>1</sup> describing this work have appeared from time to time. The object of the work described in this paper was to obtain clearer insight into the mechanism of adsorption phenomena, particularly selective adsorption, and the characteristic effects of one solute upon the adsorption of another.

It is a well-known fact that either by leaching or by shaking a soil with a solution of potassium chlorid (or some neutral salt) the amount of potassium present will be diminished, and a certain amount of the bases of the soil (Ca, Mg, etc.) will be found in the resulting solution, while the amount of the chlorin will remain practically unchanged. Also, the resulting solution is slightly but distinctly acid to our common indicators.

On treating kaolin with solutions of magnesium and sodium chlorids Kohler<sup>2</sup> found the resulting solutions to be slightly but distinctly acid

<sup>1</sup> Cameron, F. K., and Bell, J. M. The mineral constituents of the soil solution. U. S. Dept. Agr., Bur. Soils, Bul. 30, 1905.

Cameron, F. K., and Patten, H. E. The distribution of solute between water and soil. Jour. of Phys. Chem., v. 11, p. 581-593, 1907.

Patten, H. E. Some surface factors affecting distribution. Trans. Amer. Electrochem. Soc., v. 10, p. 67-74, 1906.

Patten, H. E., and Gallagher, F. E. Absorption of vapors and gases by soils. U. S. Dept. Agr., Bur. Soils, Bul. 51, 1908.

Patten, H. E., and Waggaman, W. H. Absorption by soils. U. S. Dept. Agr., Bur. Soils, Bul. 52, 1908.

Schreiner, Oswald, and Failyer, G. H. The absorption of phosphates and potassium by soils. U. S. Dept. Agr., Bur. Soils, Bul. 32, 1906.

<sup>2</sup> Kohler, Ernst. Adsorptionsprozesse als Faktoren der Lagerstättenbildung und Lithogenesis. Ztschr. Prakt. Geol., Jahrg. 11, p. 49-59, 1903.

to litmus and attributed this to the fact that a selective concentration of the dissolved substance—an adsorption of the base—had taken place.

E. C. Sullivan<sup>1</sup> repeated these experiments and obtained the same result, accounting for it by an exchange of the sodium and magnesium of these salts in part for the iron and aluminium of the kaolin, the salts of the latter undergoing extensive hydrolysis in dilute solution.

Similarly, the acidity of a salt solution after treating a soil with it is explained by some as a hydrolysis of aluminium and iron salts after the replacement by the base of the salts and by others as a selective adsorption of the base of the salt.

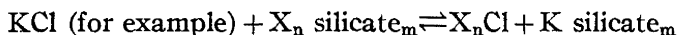
It has been found by many experimenters that on quantitatively determining the replaced bases present in a salt solution after treating a soil, kaolin, various silicates, etc., with the solution the replaced bases are equivalent or very nearly equivalent, within the limits of experimental error, to the loss of the base of salt.

Van Bemmelen<sup>2</sup> treated 100 grams of soil with 200 c. c. portions of solutions containing 8 and 40 mg. equivalents of potassium chlorid. After filtration the solutions were analyzed, and it was found that an almost complete exchange of potassium for sodium, calcium, and magnesium had taken place. Chlorin was determined in one experiment and had not changed.

Sullivan<sup>3</sup> found that by treating kaolin and various other silicates with salt solutions a quantity of bases almost equivalent to the loss of the base from the salt was dissolved in each case.

Wiegner<sup>4</sup> found that on treating an artificial amorphous water-containing (hydrated) so-called double silicate with a neutral salt solution the cation of the neutral salt was taken in part from the solution, and in its place the cations of the silicate-gel in nearly equivalent amounts entered the solution. The anion of the neutral salt remained unchanged, provided secondary reaction did not take place.

From many similar investigations with the same general result—namely, that the bases dissolved are very nearly equivalent to the loss of the base of the salt in solution—it would seem and is concluded by many experimenters that an exchange of bases takes place in the soil according to the following reaction:



From the standpoint of fertilizer practice, however, on applying potassium chlorid to the soil it is very unlikely that the above reaction takes place and that the potassium is held in the soil as a relatively insoluble silicate and in a form highly unavailable for plants.

<sup>1</sup> Sullivan, E. C. The interaction between minerals and water solutions. U. S. Geol. Survey, Bul. 312, 1907.

<sup>2</sup> Bemmelen, J. M. van. Das Absorptionsvermögen der Ackererde. Landw. Vers. Stat., Bd. 21, p. 135-191, 1877.

<sup>3</sup> Sullivan, E. C. Op. cit.

<sup>4</sup> Wiegner, Georg. Zum Basenaustausch in der Ackererde. Jour. Landw., Bd. 60, p. 111-150, 197-222, 1912.

Certain inactive solid substances presenting large surfaces have the power of taking salts from solution—that is, what is known as absorbing or adsorbing them, a phenomenon most logically explained at present as a concentrating of the solute at the surface of the adsorbing material. Qualitatively, it is known that certain of these inactive solid substances not only have the power of adsorbing a neutral salt from its solution as a whole, but may adsorb one ion more than the other, or selectively adsorb. In so doing, a partial hydrolysis of otherwise practically unhydrolyzed salts is brought about, since the removal of one ion of the salt more or at a greater rate than the other takes an equivalent number of ions of opposite charge from the water and thus leaves an excess of either hydrogen or hydroxyl ions in the solution. That such is the case can be shown by the use of common indicators, after shaking solutions of neutral salts with or percolating them through certain of these inactive solid substances.

These cases are so numerous that only a few of the best known and more convincing ones will be here recalled.

A silver-nitrate solution shaken with animal charcoal and the supernatant liquid filtered and tested with methyl orange or litmus gives a distinct color of acid reaction.

A potassium chlorid or nitrate solution shaken with cane-sugar charcoal and the supernatant liquid filtered and tested with phenolphthalein gives a strong red color of alkaline reaction.

An interesting case of selective adsorption is to be found in our common indicator, Congo red, and absorbent cotton. If the base of a column of absorbent cotton is immersed in a solution of Congo red made very slightly acid, in a very few minutes the cotton immediately above the solution is colored blue (acid reaction), while above the blue color for about an inch in height is seen the red color of neutral or alkaline reaction; above the red the cotton is wet with water.

The soil possesses all the essential properties of these adsorbing materials; but that it has the power of selectively adsorbing to any appreciable extent has for a long time been a question of dispute. The fact that a solution of a neutral salt after contact with a soil is as a rule distinctly acid to indicators supports this hypothesis.

If a soil in contact with a solution of potassium chlorid adsorbs potassium ions at a much greater rate or in greater proportion than chlorine ions, thereby (since an equivalent number of hydroxyl ions are also removed with the potassium ions) causing a partial hydrolysis of the solution ( $\text{KCl} + \text{HOH} = (\text{KOH}) \text{ adsorbed} + \text{HCl}$ ), then free hydrochloric acid will be left in the solution.

It is not unreasonable to assume that the uncombined acid might dissolve an almost equivalent amount of bases from the soil particles. On this assumption, by using a solution of a salt of potassium with a weaker acid than hydrochloric, there should be a greater adsorption of potassium ions,

since the salt is more easily hydrolyzed than potassium chlorid, less surface energy being required to obtain potassium ions from solution, while the quantity of anions adsorbed will depend upon the specific properties of the anion employed. Also, if the anion of the salt is that of a weaker acid than hydrochloric and is not adsorbed to a much greater extent than chlorin ions, a smaller amount of bases should be dissolved from the soil and a correspondingly greater acidity of the solution should result. Again, if a reaction is interposed so that the free acid will be used up before it has a chance to react with the soil particles—i. e., by adding a small amount of sodium hydroxid, yet enough to neutralize the acid theoretically set free—little or no dissolved bases of the soil should be found in the resulting solution.

On the assumption that certain ingredients of the soil adsorb in part the base of a neutral salt in solution and that the free acid resulting from the hydrolysis caused by this adsorption reacts with certain of the soil particles and dissolves an almost equivalent amount of bases of the soil, the following experimental work is based.

#### SERIES No. 1

In series No. 1, 500-gram portions of a Durham sandy loam were introduced into a number of bottles of 2-liter capacity. To the first was added 2,000 c. c. of a solution containing 7.65 grams of potassium chlorid per liter; to the second 2,000 c. c. of a solution containing potassium acetate equivalent to 7.47 grams of potassium chlorid per liter; to the third 2,000 c. c. of water. The bottles were shaken frequently at room temperature for two days. The soil was allowed to settle until the supernatant liquid was apparently clear. Portions of the supernatant liquid were then pipetted off, filtered, and analyzed.

The supernatant liquid from soil shaken with pure distilled water showed no appreciable presence of material dissolved from the soil, while the analyses of the supernatant liquids from soil shaken with the above solutions showed soil material present. The potassium-chlorid equivalents of the various constituents determined by these analyses are given in Table I.

TABLE I.—*Adsorption by Durham sandy loam of potassium from solutions of potassium salts.*

[Results stated in grams of potassium chlorid per 100 c. c. equivalent to constituents determined by analyses.]

Constituents by analysis.	From KCl solution.	From CH <sub>3</sub> COOK solution.	Constituents by analysis.	From KCl solution.	From CH <sub>3</sub> COOK solution.
	<i>Grams.</i>	<i>Grams.</i>		<i>Grams.</i>	<i>Grams.</i>
K before contact . . . . .	0.7650	0.7470	Mg after contact . . . . .	0.0157	0.0167
K after contact . . . . .	.6950	.6560	Na after contact . . . . .	.....	.....
Al after contact . . . . .	.0107	.0015	Free acid after contact . .	.0112	.0402
Ca after contact . . . . .	.0353	.0314	Anions after contact . . .	.7647	.7450

In the foregoing experiments the determination of the free acid is unreliable, considering the fact that no indicator could be used for titrating which was sensitive enough and at the same time unaffected by carbon dioxide. The results can be considered only as approximations. Boiling to remove the carbon dioxide is impossible when potassium acetate is used, since it hydrolyzes on boiling, giving an alkaline reaction to indicators. Iron and titanium were determined in several cases and found to be present in negligible amounts in the precipitated alumina. The amount of chlorine present in the solution was found to be practically unchanged.

From the data obtained when the potassium chloride is used, the amount of potassium chloride equivalent to loss of potassium ( $0.7650 - 0.6950 = 0.0700$  grams per 100 c. c.) during contact is greater than the amount of potassium chloride equivalent to the bases dissolved from the soil ( $0.0107 + 0.0353 + 0.0157 = 0.0617$  grams per 100 c. c.) by an amount ( $0.0700 - 0.0617 = 0.0083$  grams per 100 c. c.) about equal to the amount of potassium chloride equivalent to the estimated free acid ( $0.0112$  grams per 100 c. c.). When potassium acetate is used, the amount of potassium chloride equivalent to the loss of potassium ( $0.7470 - 0.6560 = 0.0910$  grams per 100 c. c.) during contact is again greater than the amount of potassium chloride equivalent to the bases dissolved from the soil ( $0.0015 + 0.0314 + 0.0167 = 0.0496$  grams per 100 c. c.) by an amount ( $0.0910 - 0.0496 = 0.0414$  grams per 100 c. c.) about equal to the amount of potassium chloride equivalent to the estimated free acid ( $0.0402$  grams per 100 c. c.).

When potassium acetate is used, the bases dissolved from the soil are 54.5 per cent  $\left(\frac{0.0496}{0.0910} \times 100\right)$  of what they would be if a complete exchange of bases had taken place, while, when potassium chloride is used, this percentage is 88.1 per cent  $\left(\frac{0.0617}{0.0700} \times 100\right)$ .

#### SERIES No. 2

In series No. 2, 250 grams of a Norfolk sandy loam were placed in a 2-liter bottle. To this was added 1,000 c. c. of a solution containing 18.38 grams of potassium chloride and about 1 gram of sodium hydroxide per liter. The bottle was shaken frequently at room temperature for two days. The soil was allowed to settle until the supernatant liquid was apparently clear. Portions of the supernatant liquid were then pipetted off, filtered, and analyzed.

Soil shaken with pure water showed no appreciable presence of material dissolved from the soil in the supernatant liquid.

The above potassium-chloride solution when shaken with soil showed a quantity of potassium chloride equivalent to the loss of potassium of 0.1520 grams per 100 c. c. and no appreciable loss of chlorine. The

amount of bases of the soil (Ca, Mg, etc.) present in the resulting solution was found to be negligible. If, however, too great an excess of sodium hydroxid is present, the resulting solution is discolored, and iron in appreciable amounts is found in the solution.

It was found that the addition of a small amount of sodium hydroxid to a solution of potassium chlorid prevented the presence of dissolved bases when the solution is shaken up in contact with a soil, and yet a loss of potassium occurred of the same magnitude as when bases were found in the resulting solution, the amount of chlorin remaining practically unchanged.

Believing the assumption previously made to have been entirely justified by the foregoing experimental work, the hope of finding the effect of concentration, size of soil particles, and presence of other substances, with special regard to substances commonly used in fertilizer practice, on the selective adsorption by soils led to the following experimental work:

#### SERIES No. 3

In series No. 3, 35-gram portions of a Norfolk sandy loam collected near Laurinburg, N. C., and a Marshall silt loam collected near Edgerton, Mo., were placed in 200 c. c. bottles with solutions of potassium chlorid containing varying quantities of potassium chlorid and a small amount of sodium hydroxid per liter. The bottles were then rotated in a thermostat at room temperature for two days. The soil was allowed to settle until the supernatant liquid was apparently clear. Portions of the supernatant liquid were then pipetted off, filtered, and analyzed, the results of the analyses being given in Table II.

TABLE II.—*Effect of concentration on adsorption of potassium from solutions of potassium chlorid by Norfolk sandy loam and by Marshall silt loam.*

Norfolk sandy loam.				Marshall silt loam.			
Quantity of KCl equivalent to the quantity of K per 100 c. c. of solution.		Loss.		Quantity of KCl equivalent to the quantity of K per 100 c. c. of solution.		Loss.	
Before contact.	After contact.	Per 100 c. c. of solution.	Percentage.	Before contact.	After contact.	Per 100 c. c. of solution.	Percentage.
<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>		<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>	
25.8550	25.6750	0.1800	0.70	11.8400	11.3500	0.4900	4.14
14.7700	14.6500	.1200	.81	10.0450	9.5700	.4750	4.73
9.1250	8.9650	.1600	1.75	6.6950	6.2450	.4500	6.72
6.2580	6.1100	.1480	2.36	4.4860	4.0420	.4440	9.90
4.7400	4.5950	.1450	3.06	2.6700	2.2400	.4300	16.11
3.1120	2.9600	.1520	4.89	1.1640	.7700	.3940	33.81
1.8380	1.7010	.1370	7.45				
.6406	.5640	.0766	11.96				
.3064	.2650	.0414	13.51				
.1283	.0960	.0323	25.18				

From the data obtained in this experiment (see fig. 1) we find that from the zero concentration of potassium chlorid, where necessarily the adsorption of potassium is zero, the loss of potassium during contact increases regularly with the concentration to a certain point and then remains practically constant, the surface of the soil particles having apparently taken up the greater part of the potassium possible at this point. The point at which the adsorption of potassium becomes practically constant is much lower in the case where a sandy loam is used than when a silt loam is used. The percentage of potassium adsorbed increases asymptotically as the concentration of potassium chlorid

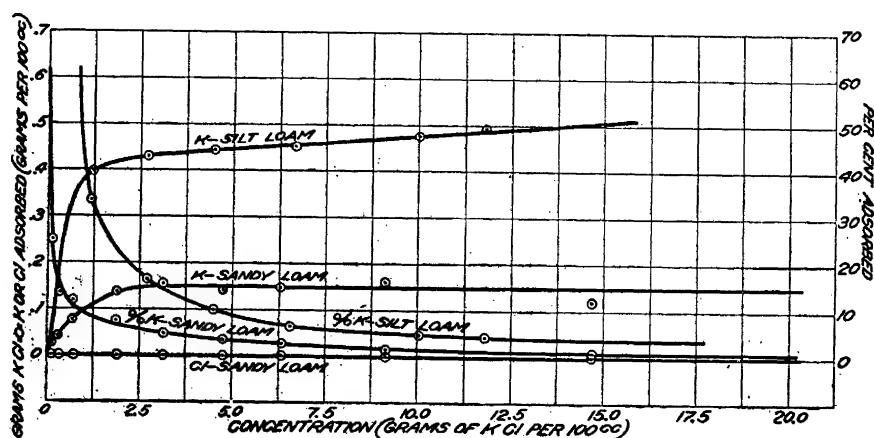


FIG. 1.—Curves showing the effect of concentration on the selective adsorption of potassium from solutions of potassium by Norfolk sandy loam and by Marshall silt loam.

decreases, and it may be concluded that the adsorption of potassium becomes practically complete at very low concentrations of potassium chlorid. Chlorin was determined in several cases and was found to have remained unchanged.

#### SERIES No. 4

In series No. 4, 35-gram portions of a subsoil of Cecil clay, a subsoil of Marshall silt loam, a subsoil of Norfolk sandy loam, a subsoil of Decatur clay loam, and a subsoil of Carrington loam were placed in 200 c. c. bottles with solutions of potassium chlorid of about the same concentration and treated as in series No. 3. The results are given in Table III.

TABLE III.—Effect of amount of surface exposed on adsorption.

Type of soil. <sup>1</sup>	Quantity of KCl equivalent to the quantity of K per 100 c. c. of solution.		Difference.
	Before contact.	After contact.	
Cecil clay.....	6. 7350	6. 4100	0. 3250
Decatur clay loam.....	6. 5550	6. 3150	. 2400
Marshall silt loam.....	6. 6950	6. 2450	. 4500
Carrington loam.....	6. 4300	6. 2050	. 2250
Norfolk sandy loam.....	6. 2580	6. 1100	. 1480

<sup>1</sup> The soils in this table are arranged in order of the relative amount of surface exposed.



As was expected, since the removal or adsorption of potassium from a potassium-chlorid solution is undoubtedly a surface phenomenon, in general the smaller the soil particles the greater was the adsorption of potassium. Clay, however, in spite of the fact that the particles are smaller than those of the other types of soil, does not show a correspondingly greater adsorptive power, the surface of the clay particles being probably of a different nature. The classification of the different types of soil is based entirely on their mechanical analysis.<sup>1</sup>

#### SERIES No. 5

In series No. 5, 35-gram portions of Marshall silt loam (the same as that used in experiment III) were placed in 200 c. c. bottles with solutions containing varying amounts of potassium chlorid per liter. To some of the portions 10 grams of sodium nitrate were added, while to others 10 grams of monobasic calcium phosphate were added. These were treated as in experiment III. A solution containing 58.25 grams of potassium chlorid per liter in contact with calcium phosphate alone lost an amount of potassium during contact equivalent to 0.0500 gram of potassium chlorid per 100 c. c. The results of the analyses of the supernatant liquids are given in Table IV.

TABLE IV.—*Effect of the presence of other substances on adsorption.*

Experiment No.	Quantity of KCl equivalent to the quantity of K per 100 c. c. of solution.		Loss.	
	Before contact.	After contact.	Per 100 c. c. of solution.	Percentage.
A.—With 10 grams of NaNO <sub>3</sub> present:				
I.....	11. 1850	10. 3750	0. 8100	7. 25
II.....	8. 9950	8. 2650	. 7300	8. 12
III.....	6. 2400	5. 6600	. 5800	9. 30
IV.....	4. 4270	3. 9470	. 4800	10. 83
V.....	2. 0450	1. 7140	. 3305	16. 15
VI.....	. 8270	. 5950	. 2320	28. 05
B.—With 10 grams of CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> present:				
I.....	11. 1100	10. 5700	. 5400	4. 86
II.....	9. 1300	8. 6200	. 5100	5. 59
III.....	6. 3400	5. 8500	. 4900	7. 73
IV.....	4. 5830	4. 1200	. 4630	10. 10
V.....	1. 9930	1. 5480	. 4405	22. 10
VI.....	. 9190	. 5500	. 3690	40. 15
C.—With 5 grams of NaNO <sub>3</sub> present:				
I.....	6. 3950	5. 8200	. 5750	9. 00
D.—With 5 grams of CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> present:				
I.....	6. 3850	5. 9000	. 4850	7. 60

Table IV and figure 2 show that the presence of sodium nitrate at concentrations of potassium chlorid below about 37.5 grams per liter

<sup>1</sup> Fletcher, C. C., and Bryan, H. Modification of the method of mechanical soil analysis. U. S. Dept. Agr., Bur. Soils, Bul. 84, 1912.

decreases the adsorption of potassium from a potassium-chlorid solution by a soil and increases it above this concentration. They also show that the presence of monobasic calcium phosphate does not alter the adsorption of potassium from a potassium-chlorid solution appreciably,

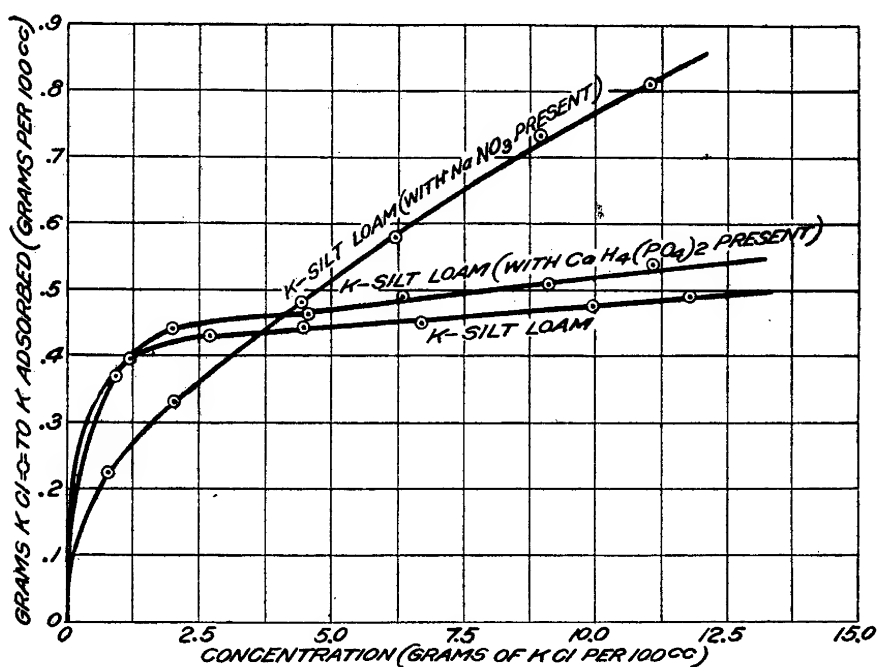


FIG. 2.—Curves showing the effect of the presence of sodium nitrate and calcium phosphate on the selective adsorption of potassium from solutions of potassium chlorid.

what change there is in the form of the curve being undoubtedly due to the removal of potassium by the calcium phosphate not in solution, either by a physical (adsorption) or a chemical reaction.

#### SUMMARY

Soils not only have the power of adsorbing dissolved salts from solutions but also of adsorbing one ion at a greater rate than the other, or selectively adsorbing, to a marked extent.

The presence of bases of the soil (Ca, Mg, etc.) in solution after shaking certain salt solutions with or percolating through a soil is probably not due to a direct chemical reaction of the salt in solution with the silicates of the soil, but to a reaction of free acid, resulting from a selective adsorption of the cation, with the mineral components of the soil.

The rate of adsorption of chlorin ions from solution by soils is much less than of potassium ions.

The selective adsorption of potassium from a potassium-chlorid solution by a soil increases in amount with the concentration up to a certain point and then remains practically constant.

The percentage of potassium adsorbed from a potassium-chlorid solution increases asymptotically as the concentration of potassium chlorid decreases and at very low concentrations adsorption is practically complete.

In general, the smaller the soil particles the greater the selective adsorption of potassium from a potassium-chlorid solution by the soil.

The presence of sodium nitrate decreases the adsorption of potassium from a solution of potassium chlorid by a soil up to a concentration of about 37.5 grams of potassium chlorid per liter and then increases it.

The presence of monobasic calcium phosphate does not change appreciably the adsorption of potassium from a potassium-chlorid solution by a soil.

Finally, if a mineral fertilizer be applied to a soil and exposed to the rain and thus dissolved and carried through the soil in solution, these substances will be adsorbed (an entirely physical phenomenon) either as a whole or selectively from the solution by the vast surface of the soil particles and will be held there by this same physical force until the plant or subsequent leaching removes it.

The presence of other mineral substances added to the soil may or may not increase or decrease the rate at which this adsorptive phenomenon takes place.

# A BACTERIUM CAUSING A DISEASE OF SUGAR-BEET AND NASTURTIIUM LEAVES

By NELLIE A. BROWN, *Assistant Pathologist, Laboratory of Plant Pathology*, and  
CLARA O. JAMIESON, *Scientific Assistant, Office of Cotton and Truck Disease and  
Sugar-Plant Investigations, Bureau of Plant Industry*

## INTRODUCTION

The bacterial disease described in this paper was first observed in the spring and summer of 1908 on nasturtium leaves growing near Richmond, Va., and on sugar-beet leaves collected from the Government plat at Garland, Utah. The disease on both hosts was of the leaf-spot type, but since the general appearance was not at all similar there was no thought at the time of a possible relationship between the causal organisms.<sup>1</sup> Investigations of the disease as it occurred on each host were at once begun, but not until the studies had progressed for nearly two years did it become evident that there was a striking similarity in regard to both cultural and morphological characteristics of the bacteria isolated from the two kinds of diseased leaves.

A comparative study of the bacteria followed, care being taken to use the same media placed under similar conditions. As a result of studies extending over four years, it has been found that in essential characteristics the bacterial organisms are so nearly identical that in the opinion of the writers the causal organism is one and the same bacterium. Any minor differences which occur may be attributed to individual adaptation due to host influence.

## OCCURRENCE AND GENERAL APPEARANCE OF THE DISEASE ON THE TWO HOSTS

The material furnishing the basis of this study was received during the spring and summer of 1908. The diseased nasturtium leaves were sent in from Richmond, Va., to Dr. C. O. Townsend, then Pathologist in Charge of Sugar-Beet Investigations in the Bureau of Plant Industry. The diseased leaves had been gathered from young nasturtium plants growing in an open garden bed and when received were somewhat wilted and discolored, showing water-soaked and brownish-colored spots from 2 to 5 mm. in diameter. Upon microscopic examination the tissue within and surrounding these diseased spots was seen to be filled with great numbers of active bacteria.<sup>2</sup>

---

<sup>1</sup> Brown, Nellie A. A new bacterial disease of the sugar-beet leaf. *Science*, n. s., v. 29, no. 753, p. 915, 1909. Jamieson, Clara O. A new bacterial disease of nasturtium. *Science*, n. s., v. 29, no. 753, pp. 915-916, 1909.

<sup>2</sup> [Halsted, B. D.] Nasturtium blight. *New Jersey Agr. Expt. Sta., 17th Ann. Rpt., [1895] 1896*, p. 410, fig. 56, 1897.

The diseased sugar-beet leaves were collected by Dr. Townsend in Utah and California on inspection trips to the sugar-beet sections of the West and were sent to the laboratory in Washington for examination. Leaves similarly diseased were also received from Oregon during the summer of 1909, but, so far as known to the writers, the trouble has not been noticed up to the present time in any other beet-growing State.

The first leaves came from Utah and had dark-brown, often black, irregular spots and streaks from 3 mm. to 1.5 cm. in diameter. They occurred on the petiole, midrib, and larger veins. Occasionally the discoloration extended along the veins for some distance, and the tissue on either side was brown and dry; sometimes there were corklike protuberances at the central point of the spots. In badly diseased petioles the tissue had softened as though affected with a soft rot, but when only a few spots occurred there was no indication of softness.

Unlike the spot diseases due to *Cercospora* and *Phyllosticta*, this spotting did not spread through an entire beet field, but was generally limited to small areas.

The tissue embracing the dark spots was examined with the microscope as soon as the material was received and was found to be filled with very active bacteria; no fungus hyphæ were seen. Some of the leaves were placed in a moist chamber and carefully watched for several days, but there was no fungus mycelium in or around the spots.

#### ISOLATION OF THE ORGANISM FROM THE TWO HOSTS

The method of isolating the bacterial organism from the diseased sugar-beet and nasturtium leaves was by means of poured agar plates. Spots from the soundest leaves were used, the tissue being immersed in mercuric chlorid (1:1,000), washed in sterile water, and mashed in bouillon. The plate colonies were up in 24 hours. They were round, thin, smooth, glistening, whitish in reflected light, bluish in transmitted light, and 1 to 5 mm. in diameter. In three days the agar in the immediate neighborhood of the colonies had changed to a yellowish-green color. No other colonies appeared on the plates.

With young subcultures from these plate colonies needle-prick inoculations were made into sugar-beet and nasturtium plants, in order to prove that the right organism had been isolated in either case. The inoculations with the separate organisms from the two hosts are as follows:

#### INOCULATIONS WITH ORGANISM ISOLATED FROM SUGAR-BEET LEAF

Inoculations with the organism isolated from sugar-beet leaves into healthy sugar-beet leaves of plants growing in the greenhouse proved that the right organism had been isolated, for in three days there were black spots at all points of inoculation. The checks were free from

spots. Some of the inoculated leaves were taken to the laboratory, the black spots examined, and numerous bacteria found swarming in the cells. From these spots, produced by the first inoculations, the organism was reisolated in pure culture, and sugar-beet leaves in the greenhouse were inoculated repeatedly, the dark spotting and streaking of the leaves occurring in every case. Altogether, more than 100 sugar-beet leaves were inoculated. Although the infection took readily at the inoculated places, the disease was not observed to occur on any uninoculated beet plants except in two instances, when several beets of a neighboring row became affected. No slugs or worms were on the leaves, but thrips were abundant, and there were also a number of grasshoppers which had escaped capture; so possibly the infection was carried by one of these insects.

When the petioles, midrib, and large veins were inoculated by means of needle pricks, the infection took very rapidly, and the discoloration often ran along the course of the veins and veinlets. When the leaf blades were inoculated at the ends of tiny veins, there was only a darkened ring around the punctures. The infection took most rapidly on the petiole. (Pl. XVII, fig. 1.) In three days after needle-prick inoculations in young growing leaves the tissue was depressed, darkened, and often ruptured for a distance of 5 mm. around the puncture. Young beet leaves with blades about 8 cm. in length very readily succumbed to needle-prick inoculation in the blade as well as in the petiole and midrib. When material from a young culture less than 2 days old was inoculated into rapidly growing leaves, the spotting began to show in 24 hours. Old tissues were also found susceptible to the disease, but the infection did not take so rapidly. The sugar-beet root also was inoculated and the disease was found to take hold there slightly. (Pl. XVII, fig. 2.) There was no soft-rot condition, but cavities occurred in the roots where the inoculation pricks were made. These cavities penetrated into the interior of the beet and reached a depth of 2 cm. within two weeks after inoculation. Occasionally a cork-like condition of a dark color followed along the immediate line of the needle prick and no cavities were present. The discoloration, however, was not nearly so dark as in the leaf, nor was there as much tendency to spread as in the leaf.

So far as the writers know, this organism has not been found in the field attacking the beet root, and as none of the field beets with affected leaves had any root trouble, it is thought that the disease in the field is confined strictly to the leaf.

Spraying the organism on the leaves of beets did not produce the disease. Precautions were taken to prevent the bacteria from drying before they had time to get into the leaves. An infection cage was placed over beets growing in the open ground in the greenhouse, the

plants were watered well, and the leaves were sprayed with sterile water and left under the cage overnight, so that the stomata would open. The following day the growth from two-day-old agar cultures was shaken up well in sterile water and sprayed on the upper and lower surfaces of the leaves. The plants were watched carefully for two weeks, but no trace of the disease was ever seen. The experiment was repeated some months later with the same result.

Some cultures were sent to Garland, Utah, and Mr. H. B. Shaw, who had charge of the experiment station there during the season of 1909, inoculated the leaves of sugar beets growing in the open field. There, as well as in the greenhouse, the plants became infected very readily. Mr. Shaw sent some of the leaves to the sugar-plant laboratory at Washington. Upon examination swarms of bacteria were found in the blackened areas. Mr. Shaw also took portions of the diseased leaves, including the spots, and inoculated other leaves with them. Fifty per cent of the leaves treated in this way became spotted.

The most striking feature of this affection as it occurs in the greenhouse from inoculations is the black color of the spots and streaks, for they stand out prominently against the green of the leaves. These leaves never become soft, but bend over at the badly sunken spots, lose their turgidity, and finally die from drying out. If the petiole is inoculated, it frequently happens that the leaf blade will drop at a sharp angle from the infected area in less than two weeks.

#### INOCULATIONS WITH ORGANISM ISOLATED FROM NASTURTIIUM LEAF

Inoculations with the organism isolated from nasturtium leaves were made into leaves of some rather old nasturtium plants growing in pots in the greenhouse. After several days small, watery-looking areas became visible, and the tissue within these areas became discolored and shriveled, resembling in all particulars the original spots from which the organism was obtained. A microscopic examination of the tissue within the diseased areas thus produced showed the cells to be filled with many active bacteria. Check plants having leaf surfaces pricked with a sterilized needle presented no indication of diseased spots. From the observation of inoculated plants it was noticed that the general appearance of the leaf spot changed considerably during the different stages of its development. Leaves of a healthy young nasturtium plant showed the effects of needle-prick inoculations within 48 hours, the tissue at first becoming slightly darker in the infected areas and presenting a water-soaked appearance. These spots gradually increased in size, becoming 4 to 6 mm. in diameter, while the tissue within became dry and brownish in color and often brittle enough to crack (Pl. XVIII). A dropping out of this diseased tissue frequently followed, and finally the whole leaf turned yellow and fell from the stem.

## REISOLATION FROM INOCULATED TISSUE

Out of a small piece of tissue cut from one of the spots produced by inoculation a bacterial organism was isolated by means of agar plates, and by careful comparison with previous cultures was found to be similar in all respects to the organism obtained from the original diseased leaves. As soon as suitable cultures of this reisolated organism could be grown, inoculations were made into healthy young plants, and again the characteristic brown and shriveled spots were produced, with an abundance of active bacteria in the tissue. By these and other similar experiments it is proved beyond a doubt that the nasturtium leaf spot is caused by a bacterial organism. The manner in which the bacteria gain entrance to the tissue of the host has not been fully demonstrated, but from observations made during the investigation it seems probable that insect injuries, as well as mechanical wounds, open the way for the entering of the parasites.

## CROSS-INOCULATIONS BETWEEN HOSTS

After proving that the right organism had been isolated from either host, inoculations into leaves of other plants were made, with the result that the sugar-beet organism proved very infectious to nasturtium, and likewise the nasturtium organism proved infectious to the sugar beet. But as the two investigators were working independently, each with one organism, this interesting fact had no particular significance at the time. Nasturtium leaves inoculated with the sugar-beet organism became spotted and watery-looking for some distance beyond the inoculation pricks, appearing in all respects similar to spots produced by inoculations with the nasturtium organism. Later, the watery-looking areas turned from a yellow to a brown color, and still later these tissues dried up and fell out (Pl. XIX, fig. 2). Some leaves drooped and died. The check leaves showed no discoloration; nor did any part of the tissue fall out, as in the inoculated leaves.

Three years afterward the same strain of the organism was inoculated into young nasturtium leaves at the same season of the year and under practically the same conditions as before, but there was a slight infection only, though young sugar-beet leaves inoculated with the same culture were badly infected.

Although inoculations with the nasturtium organism into sugar-beet leaves produced the disease, this strain of the organism was not so infectious as the sugar-beet strain on nasturtium. This difference in the behavior of the organisms in cross-inoculation was considered to be one of host influence.



## OTHER PLANTS INOCULATED WITH THE ORGANISM FROM BOTH HOSTS

That this bacterial spot is not confined to sugar-beet and nasturtium leaves has been shown by a number of inoculations performed upon other plants growing in the greenhouse. Both strains of the organism were used. Diseased spots were produced with the bacteria upon leaves of pepper, lettuce, eggplant, and upon the leaves and pods of the bean plant. Inoculation experiments were also tried on potato, clover, and daisy plants, but without any definite infection, although there was slight discoloration on potato leaves.

The stems and leaves of the young pepper plants were readily infected through needle-prick inoculations. The spots were black, and the stems seemed more susceptible than the leaves.

Lettuce leaves growing in the greenhouse blackened readily after inoculation. One plant out of seven was entirely destroyed by the infection. One month later, when the temperature of the greenhouse was not so even throughout the day and night and the plants of the same lot had stopped growing rapidly and become toughened, the organism failed to produce infection.

The leaves of eggplant were inoculated, and brown spotting resulted at the punctured places; later, these areas dropped out of the leaves.

Of these various hosts the bean proved especially susceptible to the organism, inoculations taking effect almost as readily as upon the nasturtium and sugar-beet leaves. Bean plants inoculated with a young agar culture of both strains of the organism showed the characteristic brown spots on the leaves within three to five days. Ten days after inoculation some of the diseased leaves (Pl. XIX, fig. 1) were examined, and active bacteria were found in the cells. Three weeks after inoculation the bean leaves shriveled and died. Later, inoculations which were made upon the young pods of bean plants produced conspicuous, somewhat sunken, brownish spots in the tissue. (Pl. XIX, fig. 3.)

At the same time that the inoculation experiments were being carried on, cultural and morphological studies were made with both strains in the laboratory. From time to time notes and various tests were compared, and, as a result, the identity of the two strains was established. Such being the case, only one description will hereafter be given for the two strains, except where marked differences occur.

## DESCRIPTION OF THE ORGANISM

## MORPHOLOGICAL CHARACTERS

**VEGETATIVE CELLS.**—The organism is a medium-sized schizomycete of varying length when grown in different media. It is a short rod with rounded ends, occurring singly or in pairs (fig. 1); occasionally it occurs in long chains of two to many elements and again in long unsegmented

filaments (fig. 2, *a* and *b*). In stained tissue of the hosts the average measurement of a single rod is  $1.2$  by  $0.6\mu$ . The organism grown in a 3-day-old beef bouillon culture and stained in carbol fuchsin has an average size of  $2.1$  by  $0.7\mu$ . When stained with Loeffler's flagella stain, the average is  $3.2$  by  $1.3\mu$ .

**PROCESS OF CELL DIVISION.**—Cell division takes place in the bacterium by simple, transverse fission. In order to study the process of fission, agar hanging blocks containing the organism were made in the following manner:

Thin beef-agar plates were poured and transfers from a bacterial culture streaked across the surface of the hardened agar. Agar blocks a few millimeters square were then cut out along the streak and transferred to clean cover slips. Care was taken to place the upper surface of the block next to the glass, after which the whole was turned over a Van Tieghem moist cell and kept at room temperature. At the end of 18 hours, by means of a microscopic examination of the agar block, with 2 mm. objective (oil immersion) and No. 6 ocular, bacteria were selected and their development through several generations was observed (fig. 3).

**FLAGELLA.**—The organism is

motile by means of polar flagella, varying from one to several at each pole. In general, the number is one to two, but occasionally three occur. The best results in staining flagella were obtained by the use of Loeffler's stain, with acid mordant correction. Five drops of sulphuric acid (the acid of such dilution that 1 c. c. is neutralized by the same amount of 1 per cent sodium hydroxid) were added to 15 c. c. of mordant. The flagella are threadlike, frequently wavy and somewhat tapering, often forming a loop or coil at the distal end, and are about twice as long as the body of the bacterium, actual measurement of 10 flagella giving an average of  $4\mu$  (fig. 4).



FIG. 1.—*Bacterium aptatum* from a 2-day beef-bouillon culture stained with carbol fuchsin.

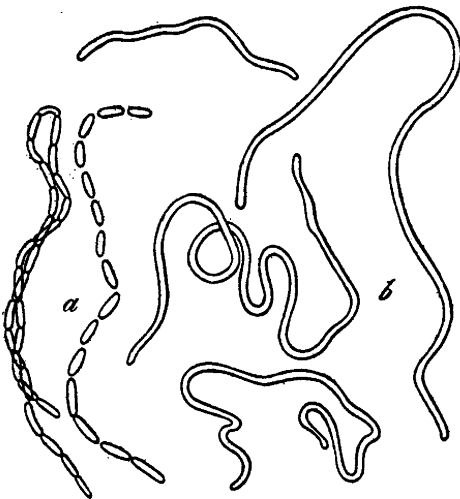


FIG. 2.—Filaments of *Bacterium aptatum* taken from the condensation water from a 2-day-old agar culture; stained with carbol fuchsin: *a*, Segmented; *b*, unsegmented.

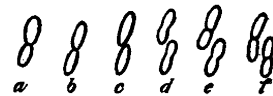


FIG. 3.—Process of cell division as seen in an 18-hour-old hanging drop culture of *Bacterium aptatum*. Time, *a* to *f*, 52 minutes.

**QUESTION OF ENDOSPORES.**—No spores have been demonstrated either by staining or testing with heat. Vacuolated forms were seen in cultures stained with spore stains. Several tests with heat were made, bouillon cultures 2 to 6 months old being treated as follows: Two were boiled three minutes and two were kept at 80° C. for 20 minutes; then transfers were made from both sets. These transfers were watched for nearly a month, but no trace of growth was seen. The transfers made before heating, as checks, showed a vigorous growth of the organism in two days. From these results it appears that spores are not formed by this bacterium, since, if present, they would have been carried over after the death of the vegetative cell, and growth would have been apparent in the new transfers. The fact that the bacterium is quite easily killed by

atmospheric drying points to the same conclusion in regard to the absence of spores.

**INVOLUTION FORMS.**—Involution forms are not common, but a few Y-shaped and cross-shaped forms were noticed in old cultures grown in media not favorable for the best development of the organism, such as beef bouillon containing 0.2 per cent of tartaric acid, or beef bouillon containing 0.1 per cent of oxalic acid. Some were found in ordinary media which had been placed under unusual conditions.

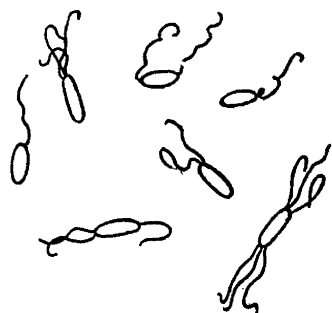


FIG. 4.—*Bacterium aptatum* showing flagella from a 2-day-old agar culture; stained with Loeffler's flagella stain.

**CAPSULES.**—No capsules have been demonstrated. The organism is viscid after growing three days on agar and five to seven days in bouillon. Ribbert's and Richard Muir's capsule stains were used.

**ZOOGLÆÆ.**—Pseudozooglææ occur in +15 bouillon, Fermi's solution, bouillon containing salt, acids, and alkalies, and other liquid media in which the growth rises in a viscid swirl when the tube is shaken. When examined under the microscope, the viscid mass is found nearly always to be made up of short rods held in place by a network of gelatinous threads. Sometimes the mass is composed largely of the unsegmented filamentous bacteria (fig. 2, b).

#### BEHAVIOR TOWARD STAINS

The organism stains readily and uniformly in the ordinary basic aniline stains, such as methyl violet, gentian violet, safranin, dahlia, fuchsin, and carbol fuchsin. It is not acid-fast and does not stain by Gram.

#### CULTURAL CHARACTERS

In general, the organism grows well upon many different kinds of artificial media, the most favorable for rapid and prolonged growth being +15 beef agar and bouillon, upon which it has been observed to live from 9 to 12 months.

**AGAR PLATES.**—At a temperature of 20° to 24° C. the colonies on peptonized beef agar (+15 on Fuller's scale) are up in 24 to 36 hours when plates are poured from a young bouillon culture. They are round, smooth, flat, glistening, 1 to 2 mm. in diameter, with entire edge, fish-scalelike markings, whitish in reflected light, bluish in transmitted light. In three days the colonies are 4 to 5 mm. in diameter on plates thinly sown, and the agar has changed to a faint yellowish green color. In 7 to 10 days the colonies are a deep-cream color.

**AGAR STROKE.**—There is a moderate growth along the stroke in 24 hours. It is whitish, flat, smooth, and glistening, spreading at base. In two days there is a heavy growth; in four days the agar has changed to a slight yellowish green, with the growth of a viscid consistency. In from five to seven days the bacterial growth covers nearly the entire surface of the agar, densely clouds the condensation water, and becomes slightly malodorous. The greatest growth of the organism occurs at the base of the stroke and in the condensation water. The margin of the stroke is often scalloped, with some edges of the scallop thinner than others. The growth on old cultures is a deep-cream color, the medium having become brown.

Tests made with the same organism, transferred at intervals for several years to artificial media, showed that the greenish color was not always produced in agar.

**AGAR STAB.**—Growth is slow in stab culture, only a slight trace occurring in two days. It is best at the surface; very little along the line of puncture. In four days the entire surface of the agar is covered with a whitish, smooth growth, and the agar at the top has changed to a faint yellowish green. Many crystals occur in the path of the needle. The agar is not liquefied or softened.

**BEEF-BOUILLON CULTURES.**—A slight clouding is noticeable in beef-bouillon (+15) cultures within 18 to 22 hours at room temperature (22° to 25° C.), increasing in density until a thick, viscid sediment forms in the bottom of the tube. When shaken, this sediment rises in a thick coherent, ropelike swirl. In bouillon cultures of three to five days' growth the solution becomes slightly greened, and a thin, whitish pellicle forms on the surface. This pellicle, which is composed of small masses of bacteria, is easily disturbed when shaken and falls in hundreds of tiny particles. In two weeks the medium has nearly cleared, a thick, whitish sediment has accumulated, and the solution is apple green in color, the fluorescence being most distinct toward the surface. In two months the medium has changed to a dark-amber color (Ridgway's "tawny"). Crystals may or may not occur.

**NEUTRAL BEEF BOUILLON.**—Growth occurs in 22 to 24 hours. There is a good growth in five days, and the medium has become a faint yellowish green color.

**BOUILLON CONTAINING SODIUM CHLORID.**—Growth occurred in neutral bouillon containing 5 per cent of sodium chlorid when tests were made with the organism soon after first isolating. Three years later these tests were repeated. Growth then took place in bouillon containing 3 and  $3\frac{1}{2}$  per cent of sodium chlorid, but there was no growth in bouillon to which 4 per cent of sodium chlorid was added.

**BOUILLON OVER CHLOROFORM.**—There is retardation of growth for two days; then the bouillon clouds and in nine days is colored a yellowish green tinge, as in the +15 bouillon without chloroform.

**NITRATE-BOUILLON CULTURES.**—In nitrate bouillon a thin clouding is produced within 24 hours, and in four days the solution is distinctly clouded, especially in its upper portion, where pseudozooglœlike masses are visible. In eight days the thin pellicle which forms on the surface is easily shaken into many small particles. At this time a slight greenish cast appears in the solution. The same ropelike sediment described in beef bouillon was observed in a 9-week's-old culture of nitrate bouillon.

**USCHINSKY'S SOLUTION.**—In plain Uschinsky's solution and in the peptonized solution (1 per cent) strong clouding was produced in three to five days. In four days a thin pellicle composed of pseudozooglœlike masses was observed. A greenish fluorescence became visible in five to eight days, and in three weeks the uniformly clouded solution had turned pale green (No. 328B, Code des Couleurs, Klincksieck et Valette).

**FERMI'S SOLUTION.**—There is a slight clouding in one day. In five days there is a thick tenacious pellicle, and the medium has changed to a decided pea-green color. A few fragments on the underside of the pellicle are suspended in the medium, and these occur in long gelatinous strings. On shaking the culture it is difficult to break up the pellicle and cause it to sink. In one month this pellicle is from 3 to 4 mm. thick.

**COHN'S SOLUTION.**—The organism does not grow in Cohn's solution.

**STERILE MILK.**—The milk is cleared slightly in two to four days, showing a gradual separation of whey from curd. This separation begins on the surface as a watery band and gradually extends downward, becoming complete in 12 days when kept at room temperature from 18 to 22° C. The medium is a yellowish cream color with a suggestion of green. There is a slight rim, but no pellicle. In one month the medium has become darker, and the green tinge has disappeared. It is translucent throughout. Compared with Ridgway's Color Chart, it is a clay color. After two months at room temperature the cultures are dried down 5 c. c., and are of a thick, creamy consistency. Transfers from these cultures showed that the organism was still alive.

**LITMUS MILK.**—In two days a blue ring appears at the surface of the liquid, extending down about 1 cm. In four days there are three rings

of graded shades of blue, while the lowest third of the liquid remains the color of the check tubes. Six to eight days later none of the original color of the liquid remains. Some tubes have four or five rings of color, the upper ring being the darkest blue. From 12 to 15 days after inoculating a brownish color appears at the bottom of the tube and extends upward, changing the entire liquid to a muddy blue in from three to six days. About four days later the medium begins to change to blue again and in seven days is entirely blue, approaching Ridgway's plum purple. Four different tests were made in which the color changes followed in this same manner. Room temperature, 18° to 22° C.

**GELATIN PLATES.**—Colonies of the bacterium which appear on gelatin (+10) plates within 48 hours are whitish, round, and glistening, with a smooth, flat surface having fishscalelike markings. Slight liquefaction began in two days at a temperature of 20° to 22° C., causing small clear areas around the colonies. In thickly sown plates liquefaction proceeded rapidly, becoming complete in three to five days. In plates thinly sown the liquefaction is only in cuplike areas about the colonies. When liquefied, the gelatin becomes a turbid, slightly greenish fluid.

**GELATIN STAB CULTURES.**—In gelatin (+10) stabs, growth was visible in two days on the surface about the stab, extending downward about 1 cm. (temperature 20° to 22° C.). Craterlike depressions with fluid contents were observed on the third day, increasing in size until a layer of fluid was formed. In 10 days this layer had become 1 cm. in depth. Liquefaction of the gelatin stab culture was complete in 30 days.

**STEAMED POTATO CYLINDERS.**—In three days growth on this medium is abundant, flat, smooth, cream white, and glistening. The potato changes to a gray-brown color in 3 days, and in 15 days is from two to four shades darker. The bacterial slime approaches Ridgway's wood brown. There is no diastasic action of the starch.

**STARCH JELLY.**—Growth is scant on starch jelly. In seven days the medium at the surface and about 3 mm. below the streak along which the growth of the organism has taken place has changed to a delicate green. The test for sugar with Fehling's solution was negative.

**LOEFFLER'S BLOOD SERUM.**—The growth is moderate and slow, scarcely a trace occurring in three days. The medium becomes gray and at the end of 32 days has liquefied a little. The stroke is filiform, flat, glistening, and smooth. The heaviest growth occurs in the condensation water.

**LITMUS-LACTOSE AGAR.**—Copious growth developed within two weeks in litmus-lactose agar cultures. The condensation water first clouded, after which growth began to show at the base of the stroke. In eight days there was growth along the entire stroke, with a spreading at the base and a pellicle formation in the condensation water. The medium was blued. At the end of nine weeks the growth was azure blue in color (No. 401, Code des Couleurs, Klincksieck et Valette).

**GENTIAN-VIOLET AGAR.**—Growth of the bacterium on gentian-violet agar was very slow, no growth being visible in 4 days and only a slight growth in 18 days. When examined four weeks after inoculation, however, a thin bluish growth was observed along the stroke and spreading from the base over the surface of the slant. The medium had paled, some of the violet color having been extracted by the bacterium in its growth.

#### OTHER CULTURAL FEATURES OF THE ORGANISM

**NITRATES.**—Nitrates are not reduced. Tests were made with nitrate bouillon cultures 5 and 10 days old in the following manner: 1 c. c. of a potato-starch solution was added to each culture, then 1 c. c. of a fresh potassium-iodid solution (1:250), after which 5 drops of dilute sulphuric acid (2:1) were added. There was no change of color in any of the 5 or 10 day old cultures.

**INDOL.**—No indol is present in cultures 1 to 10 days old. It is present, however, in cultures 11 to 25 days old. The tests were made as follows:

Transfers were made from a 2-day-old bouillon culture to Uschinsky's solution containing 2 per cent of peptone. These cultures grew at room temperature, 20° to 24° C., tests being made at the end of 1, 3, 5, 8, 10, 11, 12, 13, 15, and 25 days. Ten drops of concentrated sulphuric acid were added to each culture to be tested and after standing for five minutes, 1 c. c. of a 0.02 per cent solution of sodium nitrite was added. If no pink color was visible in the cultures five minutes after adding the nitrite, the tubes were heated to a temperature between 70° and 80° C. The rose color which indicates the presence of indol was not present in any of the tests up to the tenth day.<sup>1</sup> Indol was present in some of the 11-day cultures, but in the 15-day and 25-day cultures each one gave the definite rose-color reaction.

#### TEST FOR HYDROGEN SULPHID

No hydrogen sulphid is produced. Litmus-lactose agar slants were inoculated from a 2-day beef-agar culture. Small strips of filter paper previously moistened in a saturated solution of lead acetate were inserted in the tubes, being held in place by means of cotton plugs in such a manner as to prevent contact with the medium. In two days there was growth along the entire stroke, accompanied by a bluing of the agar, but without any discoloration of the filter paper. In six days the bacterial growth had become abundant, spreading at the base of the stroke and filling the condensation water. During a period of four weeks there was no evidence of hydrogen sulphid. The test was repeated with litmus-lactose agar, beef agar, and beef-bouillon cultures with the same result.

<sup>1</sup> In a few instances a faint pinkish color appeared on the tenth day in tests made with the nasturtium strain of the organism.

## TEST FOR AMMONIA

The organism produces ammonia. Beef-bouillon cultures (2 to 8 weeks old) were tested with Nessler's solution. Strips of filter paper were moistened with the solution and suspended in the tubes to be tested. The cultures were then heated in a water bath. A brownish red color appeared on the filter paper and in the drops of distillate which collected on the sides of the tube. This coloration indicated the presence of ammonia in the cultures. A second test for ammonia was made by placing 25 c. c. of the Nessler's solution in large-sized tubes. Ordinary test tubes of beef bouillon inoculated with the bacterium were put into these larger tubes. The inner tubes were left open and the outer tubes closed with cotton plugs. After five days a brownish precipitate had formed in the Nessler's solution, forming a ring on the glass tubes at the surface of the liquid. Check tubes used in both tests did not show this precipitation.

## TOLERATION OF ACIDS

Toleration of acids by the bacterium was tested in different percentages of tartaric, oxalic, and hydrochloric acid made up in beef bouillon. The organism was transferred from bouillon to acid cultures ranging from 0.1 per cent to 0.3 per cent solutions. Clouding occurred in 1 day in the tartaric acid in a 0.2 per cent solution, but there was no clouding in 10 days in a 0.3 per cent solution. In a 0.1 per cent solution oxalic acid there was slight clouding in 1 day, moderate clouding in 2 days, and strong clouding in 3 days, but no clouding in a 0.2 per cent solution. In the 0.1 per cent solution of hydrochloric acid, growth was slow in appearing; the solution became turbid in 1 to 2 weeks, and a greenish color was produced in the medium. No growth occurred in 0.125 per cent solution of hydrochloric acid during 10 days. A final test for acid toleration was made in beef bouillon containing hydrochloric and tartaric acids (titrating on Fuller's scale from +19 to +35). Results of this test showed heavy clouding in 5 days in +30 beef solution of both hydrochloric and tartaric acids, while no trace of clouding appeared in the +35 acid bouillons during 4 weeks.

**TOLERATION OF SODIUM HYDROXID.**—The toleration of sodium hydroxid by the bacterium is moderate. Transfers from a 7-day beef-bouillon culture clouded —15 beef bouillon in 1 to 2 days, —18 in 10 days, and occasionally a slight growth occurred in —20 after 2 weeks, but there was no clouding in —25 beef bouillon during a period of 4 weeks.

**OPTIMUM REACTION FOR GROWTH IN BOUILLON.**—The optimum reaction for growth in beef bouillon is between +15 and +30; the organism grows nearly as well at +25 as at +15, and the medium becomes fluorescent as in +15.



**GAS FORMATION.**—The organism is aerobic and does not form gas. Tests were made in fermentation tubes with water containing 2 per cent of Witte's peptone to which was added 1 per cent of each of the following carbon compounds: Glycerin, saccharose, mannite, maltose, dextrose, and lactose. (Levulose and galactose were used in addition with the strain of the organisms isolated from nasturtium.) No gas formed in any of the tubes. Because of differences between the two strains in regard to the clouding of solutions in the closed end of some of the fermentation tubes, the results of the tests are given separately.

With the organism isolated from sugar beet there was a heavy growth in the open arm of the tubes, but none in the closed ends. Dextrose and saccharose gave an acid test with litmus after the organism had been growing in the tubes 16 days. Glycerin, mannite, maltose, and lactose gave an alkaline test.

From inoculations with the organism isolated from nasturtium the following readings were made after 5, 10, and 28 days:

TABLE I.—*Readings from fermentation tubes inoculated with the nasturtium strain of the bacterium.*

Peptonized water with 1 per cent solution of—	After 5 days.	After 10 days.	After 28 days.
Lactose.....	Solution clouded in open end.	Clouded in open end and outer two-thirds of U tube; sharp line of demarcation; perfectly clear in closed end; no pellicle; litmus test, alkaline.	Clouded in open end and outer part of U tube; whitish precipitate; no growth in closed end; litmus test, alkaline.
Levulose.....	do.....	Clouded in open end and outer two-thirds of U tube; clear in closed end; no pellicle and no flocculence; litmus test, alkaline.	Clouded in open end and outer U tube; clear in closed end; litmus test, alkaline.
Maltose.....	do.....	Uniformly clouded in open end and outer two-thirds of U tube; sharp line of demarcation; no pellicle; clear in closed end; litmus test, alkaline.	Clouded in open end, with whitish precipitate; no growth in closed end; litmus test, alkaline.
Mannite.....	do.....	Clouded in open end and in U tube; no sharp line of demarcation; no pellicle; perfectly clear in closed end; litmus test, alkaline.	Clouded in open end and U tube; clear in closed end; no pellicle; whitish precipitate; litmus test, alkaline.
Glycerin.....	do.....	Uniformly clouded in open end and outer two-thirds of U tube; sharp line of demarcation; no pellicle; clear in closed end; litmus test, alkaline.	Clouded in open end, with whitish precipitate; clear in closed end; litmus test, alkaline.
Dextrose.....	do.....	Uniform clouding in open end and whole of U tube; no pellicle; clear in closed end; litmus test, acid.	Well clouded in open end, with numerous small particles in suspension; closed end clear, except a slight clouding in lower end; no pellicle; litmus test, distinctly acid.
Galactose.....	do.....	Clouded in open end and in U tube; no distinct line of demarcation; faint clouding in closed end; no pellicle; litmus test, distinctly acid.	Well clouded in open end, with many small particles in suspension; clouded in two-thirds of closed end; no pellicle; considerable precipitate; litmus test, distinctly acid.
Saccharose.....	do.....	Uniformly clouded in open end and in U tube; no sharp line of demarcation; no pellicle; clear in closed end; litmus test, feebly acid.	Thinly and uniformly clouded in open end and outer two-thirds of U tube; sharp line of demarcation; clear in closed end; no pellicle; whitish precipitate; litmus test, distinctly acid.

From Table I it may be seen that growth occurs in the open end of the fermentation tube in each of the nine solutions tried, while in the closed end there is slight clouding in dextrose and a distinct clouding in presence of galactose. In the test for alkaline and acid reactions neutral litmus paper was used. As a result of this test six of the sugar solutions showed an alkaline reaction and three (dextrose, galactose, and saccharose) showed a distinctly acid reaction. No gas formation was observed in the closed arm of any of the solutions during a period of 30 days.

#### TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. The test was made as follows:

Fresh transfers were made to beef bouillon from a 24-hour bouillon culture and placed in a Novy jar containing a solution of pyrogalllic acid and sodium hydroxid (1 gram of pyrogalllic acid to 10 c. c. of a 10 per cent solution of sodium hydroxid for each 100 c. c. of air space).

The control cultures were kept under normal conditions at room temperature.

The Novy jar was waxed and clamped tightly and connected on one side to a series of wash bottles containing pyrogalllic acid and sodium hydroxid and on the other side to the exhaust. There were stopcocks to regulate the passing of the gasses through the jar. In the jar with the cultures was a fermentation tube which had its closed arm filled with water except for a bubble of air at the top. This bubble was noted as an indicator of pressure within the jar. As the oxygen was absorbed by the solution within the jar, air was allowed to pass in from the wash bottles until the bubble in the fermentation tube indicated the normal pressure. The exhaust was used to draw off the gases from the jar.

The operation was repeated several times during a period of three hours, after which the Novy jar was sealed and set aside. The atmosphere in the jar was then practically one of nitrogen. At the end of six days the cultures were taken from the jar and examined. There was no trace of clouding in the bouillon. The controls, however, showed heavy growth; in fact they were heavily clouded within two days.

This test was made a second time, the Novy jar being set up in the same way and the bouillon transfers made from a 24-hour culture as before. This time the jar was sealed for two weeks. When it was opened no growth could be detected in any of the bouillon cultures, while the controls showed the usual heavy growth after two days. The cultures which had been kept in the Novy jar were clouded heavily five days after they were removed.

#### TEMPERATURE RELATIONS

**THERMAL DEATH POINT.**—The thermal death point is  $47.5^{\circ}$  to  $48^{\circ}$  C. when transfers are made from a 24-hour bouillon culture and the inoculated tubes are kept at that temperature in the water bath for 10 minutes,

readings being taken at half-minute intervals during that time. Many tests were made, using for transfers +15 bouillon cultures 18 hours to 6 days old. When 3 to 6 day old cultures were used and kept in the water bath for 10 minutes at 51°, the organism was not killed; nor was it killed at 53° C. for the same length of time.

**MAXIMUM TEMPERATURE.**—The maximum temperature for the organism isolated from sugar beet is 35° C., while the maximum temperature for the organism from nasturtium is 33° to 34° C.

**MINIMUM TEMPERATURE.**—The minimum temperature is between 0° and -1° C. When kept at a temperature of -2° to -5° C. for five days by means of an ice and salt mixture, the organism remains alive and begins to grow after being restored to room temperature. A good growth of the organism occurs in both agar and bouillon at 11.5° C. A fair growth occurs in bouillon at 8° C.

**OPTIMUM TEMPERATURE.**—The optimum temperature is 27° to 28° C.

#### RELATION TO LIGHT

The organism is not especially sensitive to sunlight. Thinly sown agar poured plates were exposed in bright sunlight at midday in mid-winter on bags of crushed ice out of doors, half of each plate being covered with black paper to serve as a check. The test with the organism isolated from sugar beet was as follows:

Fifty minutes exposure did not kill the organism, for colonies appeared on the exposed side of these plates in two days, but no colonies appeared on those plates exposed 60 minutes. Three different tests were made. The organism isolated from nasturtium proved more resistant to sunlight, since a few scattered colonies appeared on the agar plates even after an exposure of 80 minutes.

#### RELATION TO MOISTURE

The beet organism is killed very readily by drying, even at a moderate or low temperature. When drops of a 1-day-old, well-clouded bouillon culture are placed on sterile cover glasses and kept in the dark at a temperature of 21° to 25° C. from four to five hours, growth occurs in bouillon tubes into which these covers are dropped. When kept six hours, all the organisms are dead. With 3 to 6 day old cultures treated in the same way the organism was able to withstand drying from one to three days.

#### VITALITY IN CULTURE MEDIA

This organism lives from 10 to 12 months in liquid media, such as beef bouillon, sterile milk, and Fermi's solution, when kept at temperatures varying from 11° to 20° C. Bouillon cultures may die in four months and less when the plugs in the tubes are loose and such rapid evaporation occurs that the culture dries down. This usually takes place in the

summer at room temperature, 24° to 30° C. Beef-agar cultures live from 4 to 10 months, depending upon the temperature under which they are grown. Those cultures which die in from four to five months are grown at temperatures of 24° to 30° C.

#### LOSS OF VIRULENCE

No loss of virulence was noticed in the organism isolated from nasturtium until April, 1910 (two years after the first isolation), when inoculations were made into nasturtium and bean plants growing in the greenhouse. Five days after inoculation no apparent discoloration of the tissue could be observed. This result was unusual, since in all past inoculations the diseased spots had been readily produced. After repeated inoculations had been made from cultures of the bacterium grown in beef bouillon upon agar slants and potato cylinders it became evident that the organism, which had been growing on artificial media for two years, had lost its virulence.

In the case of the organism isolated from the sugar-beet leaf, no loss of virulence was noticed until about three years after obtaining the organism, and up to that time practically every needle-prick inoculation into sugar-beet leaves proved infectious. After three years the percentage of positive results from inoculations fell off considerably, as only the youngest leaves, growing under the proper conditions of moisture and temperature, became diseased. Efforts were made in the summer of 1911 to obtain a new strain of the organism from the field, but they were unsuccessful. Later, string-bean agar was tried and proved to be a rejuvenator of the organism isolated from both hosts. After growing on this medium, the organism was almost as infectious to sugar-beet leaves and nasturtium leaves as when it was first isolated. This virulence, however, was not permanent, for in the course of a year it became much reduced.

#### BACTERIA IN CELL TISSUE

Diseased tissue produced in both hosts by inoculation was fixed, embedded in paraffin, sectioned, and stained in carbol fuchsin. Microscopic examinations of these sections showed the presence of bacteria in large quantities within the cells of the diseased tissue (fig. 5). In sections cut through the central portion of the diseased spots the walls appeared ruptured or collapsed. The cells at the margins of these ruptured places show that the bacteria are in the cells, although most of the bacteria were seen in the broken-down tissues adjacent to the sound cells.

#### NATURAL INFECTION AND CONTROL

Since practically all of the work has been done under laboratory and greenhouse conditions, there has been no opportunity to investigate the complete life cycle of this organism or to follow out the natural means of

infection in the field. This being the case, no practical methods of control have been undertaken, but in order to determine if possible something in regard to the way in which the organism gains an entrance into the tissue of its hosts, young plants were placed in infection cages in the greenhouse and the foliage sprayed with a bacterial solution until it was thoroughly wet. This solution was prepared from 5-day-old cultures of the organism. Check plants were placed in a control-infection cage and sprayed with distilled water. Examination was made at intervals of several days, but no diseased spots appeared on either the nasturtium or sugar-beet leaves during a period of 20 days. The result of the experiment suggests that infection takes place only in bruised or wounded tissue, due to insects or to mechanical injury.



FIG. 5.—Camera-lucida drawing of a portion of a cross section of sugar-beet leaf inoculated with *Bacterium aptatum*. The cells containing bacteria were next to many collapsed cells.

#### TECHNICAL DESCRIPTION OF THE ORGANISM

##### *Bacterium aptatum*, n. sp.

According to the numerical designations adopted by the Society of American Bacteriologists, the group number of *Bacterium aptatum* is 211.2322133.

Form, a short motile rod with rounded ends; flagella, bipolar; involution forms rare; no spores or capsules observed; pseudozoogloæ occur; aerobic; smooth whitish colonies on agar plate with fishscalelike markings; clouds beef bouillon in 18 to 24 hours; produces alkaline reaction in litmus milk, with a gradual separation of whey from curd; liquefies gelatin; produces ammonia; no reduction of nitrates; fluorescence greenish; no diastasic action on potato starch; grows in Uschinsky's and Fermi's solutions; indol produced after 10 days; optimum temperature 27° to 28° C.; maximum

34° to 35° C.; minimum — 1° C.; thermal death point 47.5° to 48° C.; vitality 4 to 10 months in beef agar, 10 to 12 months in bouillon, depending on temperature; good growth on litmus-lactose agar; growth much retarded on gentian-violet agar; stains readily with basic anilin dyes; not acid-fast; not stained by Gram; tolerates acids; oxalic, 0.1 per cent; tartaric, 0.2 per cent; hydrochloric, 0.1 per cent; tolerates sodium hydroxid in beef bouillon, — 18 Fuller's scale; no growth in Cohn's solution; killed readily by drying; not very sensitive to sunlight; retains its virulence 2 to 3 years; pathogenic to nasturtium, sugar-beet, and several other plants.

#### COMPARISON OF PSEUDOMONAS TENUIS WITH BACTERIUM APTATUM

While the work on *Bacterium aptatum* was being prepared for publication, Bulletin No. 167 of the Vermont Experiment Station was received,<sup>1</sup> part 3 of which contains a description of green fluorescent bacteria

<sup>1</sup> Edson, H. A., Jones, C. H., and Carpenter, C. W. Micro-organisms of maple sap. Vermont Agr. Exp. Sta. Bul. 167, p. 321-610, 14 fig., 16 pl., 1912.

occurring in maple sap. Results of a comparative study of seven representative strains of the green fluorescent sap bacteria and six known fluorescent species are given, and the group numbers of these organisms determined. Since one of these numbers, that of *Pseudomonas tenuis*,<sup>1</sup> is identical with the group number of *Bacterium aptatum*, it was found necessary to make cultural comparisons. A culture of *Pseudomonas tenuis* was obtained by Dr. Erwin F. Smith from Mr. C. E. A. Winslow, American Museum of Natural History, who stated that he had received it from Mr. Edson.

Table II shows the results of comparative tests made with *Pseudomonas tenuis* and *Bacterium aptatum*.

TABLE II.—Comparison of the characteristics of *Pseudomonas tenuis* and *Bacterium aptatum*.

Media, etc.	<i>Pseudomonas tenuis</i> .	<i>Bacterium aptatum</i> .
1. Beef bouillon.....	Rapid clouding; green fluorescence; distinct pellicle.	Clouding with green fluorescence; distinct pellicle.
2. Beef-agar stroke.....	Smooth, thin, whitish growth; medium greened.	Smooth, thin, whitish growth; medium greened.
3. Uschinsky's solution..	Strong clouding with fluorescence; pellicle formed.	Strong clouding with fluorescence; pellicle formed.
4. Nitrate reduction.....	None.....	None.
5. Indol test.....	No indol in 10-day cultures, but present in 16-day cultures.	Indol present in 10 to 12 days.
6. Hydrogen - sulphid test.	Hydrogen sulphid produced.....	No hydrogen sulphid.
7. Gelatin plates.....	A trace of liquefaction in 3 weeks on thickly sown plates.	Liquefaction begins on second day and is complete in 5 days in thickly sown plates.
8. Gelatin stabs.....	No liquefaction in 3 weeks.....	Liquefaction begins in 2 to 3 days.
9. Sterilized milk.....	Gradual thickening in 6 weeks without clearing.	Clearing begins in 2 to 3 days and is completed in 2 weeks.
10. Litmus milk.....	Alkaline reaction; color uniform throughout during 7 weeks.	Alkaline reaction; banded appearance resulting in clearing and a uniformly blue color in 3 to 4 weeks.
11. Ammonia test.....	Ammonia produced.....	Ammonia produced.
12. Pathogenicity.....	Nonpathogenic to sugar-beet and nasturtium leaves.	Pathogenic to sugar-beet and nasturtium leaves.

From results given in Table II it is evident that *Pseudomonas tenuis* and *Bacterium aptatum*, although closely related in the green fluorescent group of bacteria, do not belong to the same species. Similarity of growth occurs and was especially noticed in beef bouillon, on beef agar, and in Uschinsky's solution. *Pseudomonas tenuis*, however, clouds bouillon and Uschinsky's solution more quickly than *Bacterium aptatum*. Both organisms produce indol and ammonia. Neither reduces nitrates. *Pseudomonas tenuis* has a strong putrefactive odor not present in cultures of *Bacterium aptatum*. *Pseudomonas tenuis* produces hydrogen sulphid, while *Bacterium aptatum* does not. In sterilized-milk cultures, *Bacterium aptatum* gradually separates whey from curd, and in litmus milk this process is accompanied by changes of color, giving a distinctly banded appearance during the first week's growth. Neither the separation of

<sup>1</sup> Zimmermann, O. E. R. Die Bakterien unserer Trink- und Nutzwasser . . . Reihe 1, Chemnitz, 1890. 106 p. Also in 11. Bericht, Naturwissenschaftliche Gesellschaft, Chemnitz, 1887, 1889, p. 53-154, 1890.

Thumm, Karl. Beiträge zur Biologie der fluoreszierenden Bakterien. Arb. Bakt. Inst. Karlsruhe, Bd. 1, Heft 3; p. 291-377. [1895.]

whey from curd nor the color changes were apparent in cultures of *Pseudomonas tenuis* during a period of seven weeks. One of the most important cultural differences between these two organisms appeared on gelatin plates. *Bacterium aptatum* is a rapid liquefier, while *Pseudomonas tenuis* showed only a trace of liquefaction in three weeks, this slight liquefaction occurring only on thickly sown plates and not at all in stab cultures. The essential difference, however, between *Bacterium aptatum* and *Pseudomonas tenuis* is not so much a cultural as a physiological one. This is shown in the ability of *Bacterium aptatum* to produce diseased spots on sugar-beet, nasturtium, and bean leaves, while *Pseudomonas tenuis* is nonpathogenic to these hosts.

COMPARISON OF BACTERIUM PHASEOLI WITH BACTERIUM APTATUM

When it was observed that *Bacterium aptatum*<sup>1</sup> produced diseased spots so readily on leaves of the bean plants, the question at once suggested itself as to the relation between this organism and *Bacterium phaseoli*, the cause of the well-known bacterial blight of bean, as described and worked out by Dr. Erwin F. Smith.<sup>2</sup> The cultural characteristics of *Bacterium aptatum* were, therefore, compared with those of *Bacterium phaseoli*. As a result of this comparison it is evident that the two organisms are entirely different.

Some of the characteristic differences between the two organisms are shown in Table III.

TABLE III.—Comparison of the cultural characteristics of *Bacterium aptatum* and *Bacterium phaseoli*.

Media, etc.	<i>Bacterium aptatum</i> .	<i>Bacterium phaseoli</i> .
Beef agar (plate).....	Whitish colonies, slightly bluish in diffused light; medium greened.	Yellow colonies, smooth, wet-shining; thin, distinct margins.
Agar slant.....	Whitish, smooth, faintly blue in transmitted light; medium greened.	Smooth, translucent, yellow; slimy consistency; growth without retardation.
Potato slant.....	Cream white to wood-brown; viscid; medium browned; no diastasic action.	Copious yellow slimy growth, medium grayed; diastasic action powerful.
Litmus milk.....	Alkaline reaction; slow clearing during seven weeks.	Slow alkalinity and separation of casein from whey.
Thermal death point.....	47.5° to 48° C.	49.5° C.
Flagella.....	Bipolar; one to several.	Polar; one.
Pathogenic to—	Nasturtium, sugar beet, bean, and other plants.	Bean and lupine.
Resistance to dry air....	Few hours to several days.	27 days.
Resistance to sunlight....	80+ minutes.	30 to 45 minutes.
Color in mass.....	Whitish.	Yellow.

<sup>1</sup> This comparison was made with *Bacterium aptatum* isolated from nasturtium.

<sup>2</sup> Smith, E. F. Description of *Bacillus phaseoli* n. sp., with some remarks on related species. Proc. Amer. Assoc. Adv. Sci., 46th meeting, 1897, p. 288-290, 1898.

—— The cultural characters of *Pseudomonas hyacinthi*, *Ps. campestris*, *Ps. phaseoli*, and *Ps. stew. arti*—four one-flagellate yellow bacteria parasitic on plants. U. S. Dept. of Agr., Div. Veg. Physiol. and Path., Bul. 28, 153 p., illus., 1901.

—— Bacteria in Relation to Plant Diseases. v. 2, Washington, D. C., 1911, p. 62. (Carnegie Inst. Washington, Pub. 27, v. 2.)

## COMPARISON OF BACTERIUM XANTHOCHLORUM WITH BACTERIUM APTATUM

While investigations with *Bacterium aptatum* were in progress, attention was called to the recent work of Dr. Julius Schuster upon a bacterial decay of the potato tuber caused by *Bacterium xanthochlorum*.<sup>1</sup> From Dr. Schuster's description it was observed that in morphological and certain cultural characters this potato bacterium resembled quite closely *Bacterium aptatum*. Since both belong to the green fluorescent group of bacteria, it seemed worth while to take up a comparative study of the two organisms. Fortunately a culture of Dr. Schuster's *Bacterium xanthochlorum* was at hand, having been brought to our laboratory by Dr. H. W. Wollenweber in November, 1911. Accordingly a series of cultural tests was begun at once and continued for a period of about three months.<sup>2</sup> As a result of these tests it is evident that *Bacterium aptatum* and *Bacterium xanthochlorum* are not identical, although their appearance is quite similar upon some kinds of culture media. Table IV gives a partial record of the results obtained and will be sufficient to show the differences.

TABLE IV.—Comparison of the cultural characteristics of *Bacterium aptatum* and *Bacterium xanthochlorum*.

Media.	<i>Bacterium aptatum</i> .	<i>Bacterium xanthochlorum</i> .
+15 beef-agar plates.....	Growth less rapid than <i>Bacterium xanthochlorum</i> ; fishscalelike markings on surface colonies pronounced.	Growth more rapid and appearance of colonies more compact than those of <i>Bacterium aptatum</i> .
+15 beef-agar stroke.....	Growth less rapid than <i>Bacterium xanthochlorum</i> and greenish fluorescence not so marked.	Growth rapid and fluorescence marked.
+15 beef-agar stab.....	Growth whitish to drab color in center of nail head.	Growth pinkish colored in center of nail head.
+10 gelatin plates.....	Growth slower than <i>Bacterium xanthochlorum</i> and liquefaction does not begin so early; medium only slightly greened.	Growth and liquefaction rapid; medium distinctly greened.
+15 beef bouillon.....	Thin pellicle of pseudozoogloëlike masses; sediment a ropelike viscid swirl; fluorescence appears slowly.	Growth rapid; pellicle membranous and falling entire; green fluorescence striking.
Potato cylinders.....	Appearance similar to <i>Bacterium xanthochlorum</i> .	Growth gradual; at first creamy white, later brownish; starch not broken down.
Nitrate bouillon.....	Less rapid growth than <i>Bacterium xanthochlorum</i> ; pellicle easily breaking into small particles; fluorescence weak.	Growth rapid; pellicle membranous and breaking into fragments; fluorescence much greater than <i>Bacterium aptatum</i> .
Sterile milk.....	Slow separation of whey from curd; no distinct fluorescence; pellicle of floating islands.	Separation of whey from curd more rapid than in <i>Bacterium aptatum</i> ; pellicle more distinct; greenish fluorescence marked.
Litmus milk.....	Color of whey blue with whitish rim formed around tube above solution; pellicle not complete.	Color of whey grayish; rim above solution pink to purplish; pellicle distinct.
Ushinsky's solution.....	Clouding less dense than <i>Bacterium xanthochlorum</i> ; fluorescence moderate; pellicle composed of pseudozoogloë-like masses.	Clouding dense; pure green fluorescence; membranous pellicle.
Litmus-lactose agar.....	Growth less rapid than <i>Bacterium xanthochlorum</i> ; blue in color; medium blued; precipitate lead colored.	Growth rapid and dense; color of growth, greenish blue; medium blued; precipitate brownish.

<sup>1</sup> Schuster, Julius. Zur Kenntnis der Bakterienfäule der Kartoffel. Arb. K. Biol. Anst. Land-u. Forstw., Bd. 8, Heft 4, p. 452-492, 13 fig., pl. 5, 1912.

<sup>2</sup> The bacterium isolated from nasturtium leaves was used in these tests.



TABLE IV.—Comparison of the cultural characteristics of *Bacterium aptatum* and *Bacterium xanthochlorum*—Continued.

Media.	<i>Bacterium aptatum</i> .	<i>Bacterium xanthochlorum</i> .
Gentian-violet agar.....	Growth of streak much retarded; no growth during first 4 days; after 18 days, moderate growth; medium paled.	No retardation; copious growth in two days; blue in color; medium greened.
Fermentation tubes.....	Acid reaction in peptonized saccharose, in peptonized galactose, and in peptonized dextrose solutions.	Alkaline reaction in peptonized saccharose solution; acid reaction in peptonized galactose and in peptonized dextrose solutions.

## SUMMARY

1. The leaf-spot diseases of sugar beet and nasturtium described in this paper are due to a bacterial organism.
2. The two diseases occurred during the same summer. The causal organism was isolated in pure cultures from both hosts and proved infectious to sugar-beet and nasturtium leaves interchangeably.
3. It is proved from cultural, morphological, and inoculation tests that the organisms causing these leaf-spot diseases on both hosts are identical.
4. The organism is also infectious to bean leaves and pods, lettuce, pepper, and eggplant.
5. It probably enters the plant through wounds or by means of insect injuries and may be spread by insects.
6. The organism is a bacterium belonging to the green fluorescent group. It is proved to be different from *Bacterium xanthochlorum*, which is pathogenic to potato, and from *Pseudomonas tenuis*, which has been given the same group number.
7. It is also different from *Bacterium phaseoli*, although both organisms produce spotting of bean leaves and pods.
8. The name *Bacterium aptatum*, n. sp., is suggested.

## DESCRIPTION OF PLATES

- PLATE XVII. Fig. 1.—Sugar-beet leaves inoculated with *Bacterium aptatum*. Photographed eight days after inoculation.  
 Fig. 2.—Sugar-beet root inoculated with *Bacterium aptatum*. Photographed two weeks after inoculation.
- XVIII (colored). Nasturtium leaves showing bacterial leaf spots 10 days after inoculation with *Bacterium aptatum*. (May, 1909.)
- XIX. Fig. 1.—Bean leaves inoculated with *Bacterium aptatum* from leaf-spot of sugar beet.  
 Fig. 2.—Nasturtium leaves inoculated with *Bacterium aptatum* from leaf-spot of sugar beet.  
 Fig. 3.—Bean pods inoculated with *Bacterium aptatum* from leaf-spot of sugar beet.  
 (Inoculated Nov. 12, 1908; photographed Nov. 25, 1908.)





*E. J. Schutt*



# THE CALLIEPHIALTES PARASITE OF THE CODLING MOTH

By R. A. CUSHMAN

*Entomological Assistant, Deciduous Fruit Insect Investigations, Bureau of Entomology*

## INTRODUCTION

The notes and observations on which the present paper is based were obtained at Vienna, Va., under the direction of Prof. A. L. Quaintance, in Charge of Deciduous Fruit Insect Investigations, Bureau of Entomology, the writer having been assigned to work on the parasites of deciduous fruit insects at the Vienna laboratory in the spring of 1911.

So much has been published concerning the *Calliephialtes* parasite of the codling moth, under the names *Calliephialtes messor* Grav. and *Ephialtes carbonarius* Christ, since its introduction into California that it seemed advisable to begin the work on the project with a study of this species and its liberation on a large scale. The specimens with which the start was made were obtained from two lots of parasitized codling-moth larvæ secured in 1911 from the California State Insectary. The propagation from the first lot was unsuccessful, only three diminutive males being reared. The second lot was received in the late summer. These were reared to maturity, 15 females and a larger number of males being secured. After these had mated they were given access to codling-moth larvæ that had been compelled to spin their cocoons in strips of strawboard. The parasites oviposited very readily in the codling-moth cocoons. The progeny of these individuals did not emerge until the following spring. A large majority were lost in an attempt to force them through to early maturity in a greenhouse, where, in spite of daily soakings with water, the pupæ dried up. A few females forced to maturity in this way deposited eggs, but only males came from them. However, 21 females and 52 males were reared later from unforced material, and it was with these that the real start in the work was made in the spring of 1912.

During the season of 1912 several hundred individuals of both sexes were reared under observation from egg to maturity. The results of these observations are recorded in the following pages.

While the major part of the work was performed by the writer, it was greatly facilitated by the work of Mr. J. D. Luckett, half of whose time during the period from June 15 to September 15, 1912, was spent in assisting in this work.

## IDENTITY AND INTRODUCTION OF THE SPECIES

When the California State Horticultural Commission began its work of introducing this parasite into California in an attempt to control the codling moth, specimens were submitted to Dr. William H. Ashmead for determination. Dr. Ashmead determined them as the *Calliephialtes messor* of Gravenhorst, a species inadequately described from a single female specimen from Russia. Up to the time of the introduction into California, *C. messor* had been mentioned in literature only once since its description. This was by Taschenberg, who in 1863 recorded it as having been reared as a parasite of (*Tinea*) *Galleria mellonella*, the wax moth.

When the writer took up the work on the species, specimens reared from the codling moth in material sent to the Bureau of Entomology from Sachsen, Germany, were submitted to Mr. H. L. Viereck, who determined them as *Calliephialtes comstockii* Cress., a species described from the United States. Later, specimens reared by the writer as progeny of the specimens received from California were sent to Dr. A. Roman, of the Stockholm Museum. Dr. Roman reported that the museum had no specimens of *C. messor*, but that those sent were identical with a specimen determined for the museum by Dr. Ashmead as *C. pusio* Walsh, another species described from America. The specimen in the Stockholm Museum bears only the label "Long I." Dr. Ashmead therefore evidently determined the same thing under two specific names, one European and the other American.

## INTRODUCTION INTO CALIFORNIA

Late in 1904 Mr. George Compere, acting as an agent of the State Horticultural Commission of California, found this species attacking the codling moth in Spain. Living specimens were sent by him to California, where they were propagated and their progeny released in infested orchards. At this time the species was supposed to be *Ephialtes carbonarius* Christ, and references to it under that name have appeared in literature, but specimens from California were determined by Dr. William H. Ashmead as *messor* Grav. and the species placed in his genus *Calliephialtes*. That it is not *Calliephialtes carbonarius* is firmly established by the well-known habit of that species of attacking wood-boring insects.

In view of the uncertainty as to the specific identity of the parasite, the writer has avoided the use of any specific name in the present paper.

## INTRODUCTION INTO SOUTH AFRICA

From California specimens of the species were sent to the Cape of Good Hope in 1907, where they were propagated and released by the Government Entomologist, Prof. C. P. Lounsbury. Reports of the results of this introduction indicate that it is of doubtful success.

## DESCRIPTION OF THE SPECIES

## GENERAL DESCRIPTION

The adult female is normally about half an inch long, exclusive of the ovipositor, which about equals the body in length. It is of the characteristic pimpline appearance, long and slender, black in color, with the legs red and the membranous portions of the venter white. The ovipositor is straight for most of its length, but toward the tip curves somewhat ventrally. The male is somewhat shorter and more slender than the female, as is commonly the case in this group.

## VARIATION IN SIZE

There is considerable variation in size, depending upon the abundance of suitable larval food, a few individuals of each sex of not more than half the normal dimensions having been reared. However, extremely diminutive individuals are usually males.

## TECHNICAL DESCRIPTION

**FEMALE.**—Length 11 mm.; ovipositor 11 mm., curving slightly ventrally at the tip; abdomen about twice as long as thorax. Head and abdomen black; tegula and a small triangular spot on the dorso-posterior angle of the mesonotum pale yellow, and a very small spot on the dorsal border of the mesopleurum dark brown; thorax otherwise black; palpi pale; antennæ with two basal segments black, remaining segments dark brown; all legs uniform dark fulvous; wings slightly brownish; veins and stigma brown. Thorax finely and sparsely punctate; propodeum more coarsely and densely punctate, with a shining, impunctate, median depression; abdominal segments coarsely and densely punctate; segments 2 to 5 with a smooth, shining, impressed area on the posterior lateral angle. Sheath of ovipositor black, densely hairy; ovipositor proper brown, shining.

**MALE.**—Length 9.5 mm.; more slender; otherwise, except in sexual characters, like female.

## DESCRIPTIONS OF THE THREE SPECIES TO WHICH THIS SPECIES HAS BEEN REFERRED

**Calliephialtes messor** (Grav.).

*Calliephialtes messor* Gravenhorst was originally described in the genus *Ephialtes* in 1821 (1)<sup>1</sup> from a unique female from Russia. Dalla Torre (5) credits Gravenhorst with having recorded *Tinea mellonella* as a host of this species, but this should be accredited to Taschenberg (2).

*E. messor* n.—Pedibus rufo-fulvis, tibiis posticis arcuatis. f. (aculeo longitudine corporis).

Statura, imprimis proportione et tuberculis segmentorum, haec species medium tenet inter antecedentem et sequentem; tibiis posticis arcuatis ab utraque differt.

Longitudo fere 7 linearum. Caput palpis fulvis. Thorax puncto parvò testaceo ad radicem alarum. Alae testaceo-hyalinae, stigmatē et radio fulvis, radice et squamula stramineis, areola triangulari sessili. Pedes rufofulvi, postici tarsis fuscis,

<sup>1</sup> Figures in parentheses refer to "Literature cited," p. 235-237.

tibiis arcuatis, supra fuscentibus. *Abdomen* thorace triplo longius, eoque paulo angustius, cylindricum, segmentis 3 et 4 latitudine paulo longioribus, 5-7 quadratis, omnibus tuberculis lateralibus subprominentibus. *Aculeus* longitudine corporis, terebra badia.

Unicam feminam Besser e Volhynia transmisit.

A translation of this description is given below.<sup>1</sup>

### **Calliephialtes comstockii** (Cress.).

The only reference to *Calliephialtes comstockii* Cresson is the original description published in 1880 (4). The type was reared as a parasite of *Retinia comstockiana* Fernald. It was referred to the genus *Ephialtes*.

*Ephialtes comstockii* Cresson, n. sp.

**FEMALE.**—Black, shining; thorax smooth, very feebly punctured; metathorax smooth, rounded, with two abbreviated, longitudinal, feebly developed elevated lines on disk, slightly divergent posteriorly; tegulae white; wings hyaline, subiridescent, nervures and stigma fuscous, the latter with a pale spot at base, areolet as usual; legs including coxae bright; posterior tibiae and tarsi black; abdomen about twice the length of the thorax, distinctly punctured; sides of the second and following segments tuberculated; first segment a little longer than broad, broadly excavated at base and slightly grooved on disk above; second segment longer than broad, widened posteriorly; third and fourth segments quadrate; remainder transverse; ovipositor as long as the body; length of body .35 inch.

**HABITAT.**—Ithaca, N. Y. Parasitic upon *Retinia comstockiana* Fernald.

### **Calliephialtes pusio** (Walsh.).

*Calliephialtes pusio* Walsh was originally described in 1873 (3) in the genus *Ephialtes* without host record, this constituting the only reference to the species in literature.

*Ephialtes pusio*, n. sp.—♀. Differs from *gigas* ♀ as follows:

1. The size is 1/2 smaller.
2. The face is highly polished and scarcely punctate.
3. The metathoracic carinae are obsolete, being represented only by a slightly impressed stria extending 1/3 of the way to the tip.
4. The carinae of the first abdominal joint are entirely obsolete.
5. The relative proportions of the first 5 abdominal joints are quite different, 2-4 being equal in length and each twice as long as wide, and 1 about 1/4 shorter, and 5 a trifle shorter than 2-4.
6. The usual tubercles are obvious only on 3 and 4, and are much less prominent and round, not elongated.
7. The ovipositor is rather piceous than black.
8. The legs are pale rufous, all the sutures a little darker, but both trochanters of the front leg, and the outermost one in the middle and hind leg, are whitish; and in the front leg the tarsal tip, in the middle leg the exterior face of the tibia and the whole tarsus, and in the hind leg the extreme tip of the femur and the whole tibia and tarsus, are pale fuscous.
9. The wings are subhyaline. Length ♀ .60 inch; front wing ♀ .36 inch; length abdomen ♀ .42 inch; width abdomen ♀ .06 inch; ovipositor .85 inch.

<sup>1</sup> *E[phialtes] messor*, n. sp.—Feet rufo-fulvous, posterior tibiae arcuate, female with the ovipositor as long as the body.

In habitus, especially in proportions and in the tubercles of the segments, this species stands midway between the preceding [i. e., *E. tuberculatus*] and the following [i. e., *E. manifestator*]; in its arcuate posterior tibiae it differs from both.

Length about 7 lines. Head with the palpi fulvous. Thorax with a small testaceous spot at the base of the wing; wings testaceo-hyaline, stigma and radius fulvous, base and tegulae stramineous, areolet triangular and sessile; legs rufo-fulvous; posterior tarsi fuscous; tibiae arcuate, shading to fuscous above; abdomen three times as long as the thorax, and slightly narrower, cylindrical, segments 3 and 4 slightly longer than broad, 5 to 7 quadrate, all lateral tubercles subprominent; ovipositor as long as the body, terebra brown.

A single female sent by Besser from Volhynia.



## METHODS AND APPARATUS USED IN PROPAGATION

The most convenient and successful cage devised, the one in use at present, is constructed as follows:

A glass cylinder about 6 inches in diameter and 10 inches long is laid on its side in a baseboard constructed to keep the cylinder from rolling. The back end is covered with cheesecloth held in place by rubber bands. The front is a frame about 12 inches square, over which is tightly stretched a piece of cheesecloth. This is held against the front of the cylinder by means of rubber bands stretched between nails at the side of the frame and the side of the baseboard, permitting access to the cage without actually removing the front frame, by simply pulling the frame down, as the rubber bands will stretch sufficiently to admit the hands.

The cage is almost equally lighted from all sides, and the cheesecloth at each end permits good circulation. It is very easy of construction and management and very easily cleaned. In addition, a parasite either dropping or crawling from the top of the cage almost invariably reaches the rack of codling-moth cocoons at the bottom. About 15 adult female parasites can be placed in one cage.

The racks in which the codling-moth larvæ were placed for spinning were of two kinds, depending on the use to which the larvæ were to be put. For ordinary propagation the common corrugated strawboard used in packing glassware was used. This was cut across the corrugations into strips about three inches long and five-eighths of an inch in width. This gives comfortable quarters in each cell for a single worm. These were placed on edge in small wooden boxes 3 inches long by  $2\frac{1}{2}$  inches wide and three-fourths of an inch deep. Worms placed on the racks crawled almost immediately into the cells and shortly spun up. One box at a time was placed in a cage with the adult *Calliephialtes* for parasitization.

For the detailed study of the life history of the parasite double slides of transparent celluloid were constructed. The celluloid was cut into strips three inches by five-eighths of an inch. These were held apart and the space between divided into seven cells of the proper size by small slips of cardboard one-tenth of an inch thick and held in place by being fastened with shellac to one of the celluloid strips. The whole was held together by small gummed labels pasted over the ends. Each cell was numbered on the cardboard slip preceding it. Each slide was also given a number, and the slides used in each experiment were grouped under a Roman numeral. In this way notes on the contents of any given cell could be definitely associated with the subject without any chance of confusion. With this device it was only rarely that accurate observations on the development and activities of the insects within the cells could not be readily made by transmitted light.

When not under observation, each slide was placed in a folder of dark paper which left only one edge exposed, and was filed with others of the same experiment in a shallow box constructed for the purpose.

Observations were as a rule made twice daily, in the early morning and in the late afternoon, the intervening time being considered, for the purposes of the notes made, as half a day.

It was found that a living worm within its cocoon would respond immediately to the stimulus if a needle was thrust through the bottom of the cocoon. This aided materially in the determination of the time at which oviposition of the parasite took place, since, with but one exception, the parasite was never known to deposit an egg without first killing the host larva.

The food supplied the parasites consisted of sweet liquids, such as sugar solution, dilute molasses, and strained honey. All of these substances were lapped up greedily by the parasites of both sexes.

## REPRODUCTION

### THE EXTERNAL SEXUAL APPARATUS

**OVIPOSITOR.**—The ovipositor (figs. 1, 2, and 3) is composed of five long slender pieces. The two outer ones are black and hairy, grooved longitudinally within, and form a tube or sheath surrounding the ovipositor proper. Next inside of this is a smooth chitinized piece, deeply grooved on the ventral side and terminating in a prowlike point. At its base it is forked, indicating that it is formed of two opposed pieces fused along their dorsal edges. Within this is a pair of very slender flattened pieces barbed at their tips.

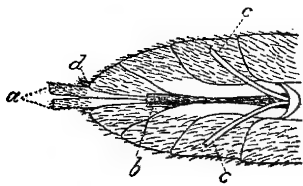


FIG. 1.—*Calliephialtes* sp.: Ventral view of terminal abdominal segments, showing relative position of elements of ovipositor. *a*, Valves of sheath; *b*, lance; *c*, lancets; *d*, cerci.

The outside pair together form the sheath. This has no part in the act of oviposition, but is merely a protection for the ovipositor proper, which is composed of the three other pieces. The single piece may be called the "lance," since it is with this that the host larva is pierced. The inner pair have been variously termed "lancets," "stylets," etc. In oviposition the egg passes down the channel formed by the three parts of the ovipositor proper.

On each side and slightly above the base of the sheath is a small tuberclelike appendage bearing a number of long, stiff hairs. These are the cerci.

**GENITALIA OF MALE.**—The male external sexual organs (figs. 4 and 5) consist of two sets of paired pieces and the penis. The outer pair are

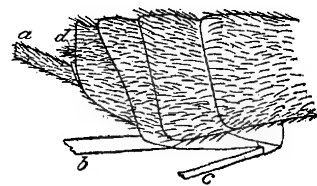


FIG. 2.—*Calliephialtes* sp.: Lateral view of terminal abdominal segments, showing relative position of elements of ovipositor. *a*, Valves of sheath; *b*, lance; *c*, lancets; *d*, cerci.

broad, tapering toward the tip, concave within, and, except during copulation, fit together like the two valves of a mussel shell, forming a sheath inclosing the other organs. They are homologous with the parts of the ovipositor sheath, and, like those, probably have no other function than that of protection for the more essential organs. The penis is probably homologous with the lance of the ovipositor, since

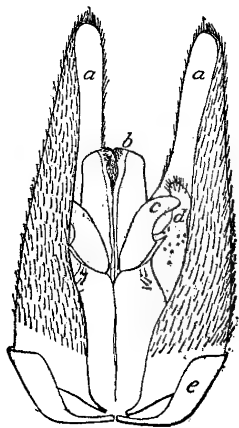


FIG. 4.—*Calliephialtes* sp.: Ventral view of male genitalia. *a*, Sheath; *b*, penis; *c*, clasper; *d*, genital palpus; *e*, cardo.

its position in relation to the other organs corresponds to that of the lance in relation to the other portions of the ovipositor. It is a fleshy, flattened organ, terminating ventrally in two lobes contiguous at their apices. Immediately in front of these on the ventral side is an opening leading into the cavity of the organ. Immediately below the penis and on each side is a 2-jointed appendage corresponding to the lancets of the ovipositor. The basal joint of this organ is thick and muscular and on the dorsolateral side is prolonged into a blunt projection bearing at its tip a number of stiff hairs. It is probably a tactile organ, and may be called the genital palpus. The second joint is a large blunt tooth which curves laterad. It probably serves the double purpose of clasper and dilator. The genitalia, as described above, are surrounded at the base by a more or less cup-shaped chitinized piece, the cardo.

#### COPULATION

Copulation occurs shortly after the emergence of the female and may evidently be repeated. The attraction between the sexes seems to be rather weak and is somewhat stronger in the female than in the male, as evidenced by the excited movement of the antennæ and wings in that sex on the approach of the male. The male apparently must be within about an inch of the female before he becomes conscious of her proximity. Of courtship there is none, the male simply jumping to the back of the female as soon as he perceives her. If she is not ready for his attentions a lively encounter ensues, the female using her hind legs and wings in freeing herself from the male. The act of copulation is short, no case having been observed in which the sexes were together more than five minutes. In copulation

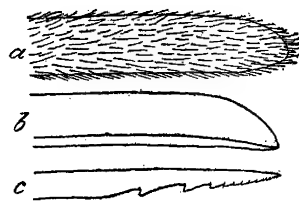


FIG. 3.—*Calliephialtes* sp.: Lateral view of tips of elements of ovipositor. *a*, Sheath; *b*, lance; *c*, lancet.



FIG. 5.—*Calliephialtes* sp.: Ventral view of clamping organ of male genitalia. *a*, Basal portion; *b*, clasper; *c*, genital palpus.

the tip of the abdomen of the male is curved down at one side of the abdomen of the female while he clings to her wings and body.

#### OVIPOSITION

Oviposition began in the cages about nine days after the emergence of the female. The stage of the host selected is the full-grown larva in its cocoon. In no case was any other stage attacked.

The act of oviposition (Pl. XX, figs. 2 and 3) was observed many times. The insect first explores the surface of the cocoon carefully with her antennæ. Then standing "on tiptoe" directly over the cocoon she raises the abdomen to a perpendicular position, at the same time lowering the ovipositor. Sometimes the ovipositor is lowered the entire distance free from the sheath, the latter remaining in line with the abdomen; but more frequently it is not released until it is at or below the horizontal, in which case the sheath bends downward, only the tip clasping the ovipositor. The sheath finally snaps back into position in line with the abdomen.

When the lowering of the ovipositor is completed it lies along the ventral surface of the abdomen and extends down between the legs, while the tip of the abdomen is bent downward over the base of the ovipositor. The tip of the ovipositor, guided by the antennæ, is placed against the surface of the cocoon. The antennæ are then extended in front of the head and almost parallel with the surface on which the insect is standing. The insect is now exactly analogous to a machine drill, the body and legs representing the machine and the ovipositor the drill. The bent-over tip of the abdomen is pressed against the base of the ovipositor, which bends forward against the ventral surface of the abdomen. With a more or less augurlike motion the ovipositor is forced through the cocoon. A few rapid jabs stir up the prospective host larva and it begins a desperate attack upon the ovipositor of its enemy, biting it and sometimes holding on with bulldog tenacity. In a number of cases the defense of the larva was so determined and powerful that the parasite was defeated and left the field minus a portion of her ovipositor, which had been bitten off by the larva. Usually, however, the parasite is successful in her efforts and finally thrusts her ovipositor into the larva, stinging it into insensibility. The stinging is usually repeated one or more times after intervals of rest. The subjugation of the host accomplished, the ovipositor is withdrawn from the host and thrust its entire length into the cocoon; then the parasite rests quietly for several minutes. In this position the abdomen is bent downward so that the tip is close to the base. The ovipositor sheath during all this time has retained its vertical position and is now in contact with the dorsal surface of the abdomen for about one-third of its length. In a few moments there begins a pulsation

of the membranous portion of the venter at the base of the ovipositor, at which time the egg is being forced into the ovipositor. The egg slips rather quickly down the ovipositor, becoming visible at a point just inside the cocoon and remaining visible during the remainder of its passage. It leaves the ovipositor, caudal pole first, at a point about 1 millimeter from the end on the ventral surface. It is placed at almost any point in the cocoon, not necessarily on the host larva.

Her egg having been deposited, the parasite usually gives a parting thrust or two and withdraws the ovipositor, which springs back into its sheath.

The duration of the act of oviposition is very variable, depending on the length of time required to locate and kill the larva. The shortest time observed was 11 minutes and the longest fully 45 minutes. The essential portions of the operation, however, probably do not require more than 4 or 5 minutes in the aggregate.

Only one egg is deposited at a time, and normally only one parasite develops on a single host. However, in a considerable number of instances superparasitism took place, and in a few cases under observation two parasites developed on a single codling-moth larva. This tendency was undoubtedly encouraged by the confinement of the cages, and as many as seven eggs were deposited in one cocoon.

No data were kept on the exact number of eggs deposited by individual parasites nor on the number deposited daily by individuals, since in each of the life-history cages from five to nine females were used. But the results in these cages indicate that the total individual oviposition was in the neighborhood of 75 eggs and the average daily oviposition about 2 eggs.

#### THE EGG

The egg (fig. 6) is opaque white, smooth, 1.5 mm. long, and about one-fifth as wide at the widest part. It is rounded at the cephalic end and tapers to a long point at the caudal end; in one plane it is considerably curved. The surface is without sculpture.



FIG. 6.—*Calliephialtes* sp.: Egg.

As the embryo develops, it draws away from the poles, and the chorion appears transparent and shriveled. Hatching takes place through a slit on one side near the cephalic pole, the larva freeing itself by a series of contortions which finally throw off the egg-shell, which is very tough and persistent.

The incubation period for 825 eggs was determined. It varied from one to seven days, depending on weather conditions. Table I shows the incubation periods by months, the number of eggs hatching in each period, and the weighted average mean temperature for each period and for the season.

TABLE I.—Incubation periods of eggs of *Calliephialtes* sp. and the relation between incubation period and temperature at Vienna, Va., 1912.

Incubation period.	Number of eggs hatching in—							Total.	Average mean temperature.
	Apr.	May.	June.	July.	Aug.	Sept.	Oct.		
1 day.....		16	4	49	17	1	.....	87	°F.
1.5 days.....		68	36	75	57	4	10	250	78.0
2 days.....		169	20	35	19	6	6	255	74.4
2.5 days.....		15	7	7	1	13	29	72	70.2
3 days.....		40	1	5	1	2	26	75	67.0
3.5 days.....		2				5	9	16	62.7
4 days.....	1	11				5	7	24	58.7
4.5 days.....		2			1			12	58.9
5 days.....	8	4			1		9	12	55.3
5.5 days.....							7	20	55.6
6 days.....	3	3					4	4	57.2
6.5 days.....							2	8	54.8
7 days.....	2							2	
Total.....	14	330	68	171	97	36	109	825	
Average.....	5.43	2.15	1.74	1.54	1.60	2.64	3.18	2.14	69.96

The relation of incubation period to the average mean temperature based on the figures of Table I is shown in graphic form in figure 7. Reference to this diagram will show that with a fair degree of constancy the duration of the incubation period varied inversely as the average mean temperature.

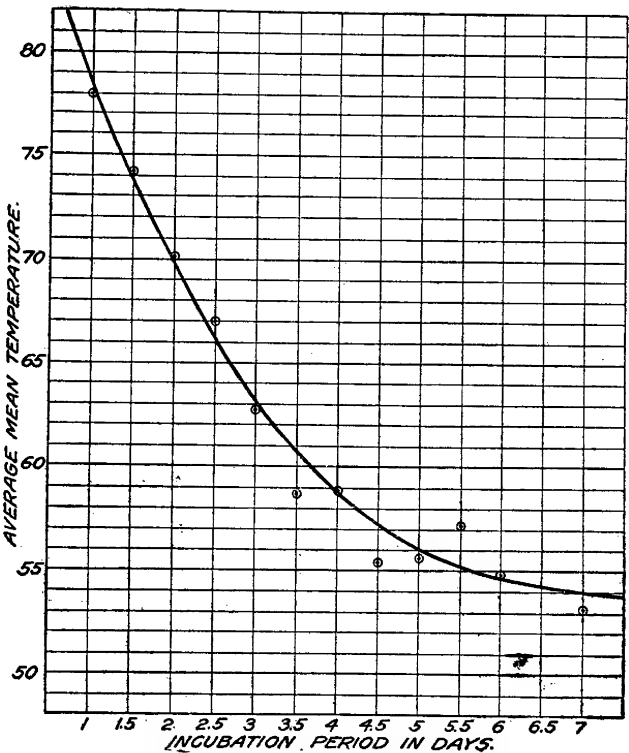


FIG. 7.—Diagram showing relation between incubation period of eggs of *Calliephialtes* sp. and average mean temperature at Vienna, Va., 1912.

THE LARVA

The newly hatched larva (fig. 8) is yellowish, slightly shorter than the egg, and widest across the head. The head is distinctly separated from the rest of the

body. The body is about three and one-half times as long as the head and is composed of 13 segments, tapering in size toward the caudal end. The head of the newly hatched larva is shown in ventral view in figure 9.

The form of the larva changes after the first molt to thick spindle shape; it is curved dorso-ventrally and is without a definite head. When full grown (fig. 10), it varies much in size, depending on the condition and abundance of food. Normally it is about three-eighths of an inch long and slightly less than a third as thick in its greatest diameter. It is pinkish white in color, the body contents showing through the transparent skin, while the adipose tissue appears as opaque-white granules. Larvæ that later develop into females average somewhat larger than those that develop into males. The face of the full-grown larva is shown, much enlarged, in figure 10, b.

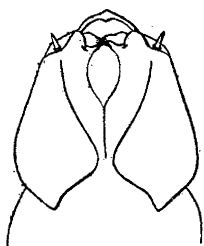


FIG. 9.—*Calliephialtes* sp.: Ventral view of head of newly hatched larva.



FIG. 8.—*Calliephialtes* sp.: Dorsal view of newly hatched larva.

The larva begins feeding very shortly after hatching and may attack its host at almost any point, although it is more likely to attack the dorsum or sides than the venter. As feeding continues, it may change its position occasionally. In most cases the point of attack is finally shifted to a point near the posterior end of the host, the parasite pushing the collapsing skin up toward the head until there is nothing left of the host but a pellet consisting of skin and head shield. This is finally pushed to one end of the cocoon.

*Calliephialtes* is normally a solitary parasite, but as indicated in the foregoing discussion of oviposition, more than one egg was deposited on numerous occasions on a single host; though on only a few occasions did more than one live beyond the first stage. Usually the extra eggs did not hatch, owing probably to their being destroyed by the first larva to hatch. The actual destruction of eggs in this way was observed on a few occasions. However, in a very few instances, two larvæ developed on a single host. In such cases neither of the larvæ attained normal size and all produced dwarf adults. In only one instance of double parasitism was an adult female produced, and then the other individual was a male.

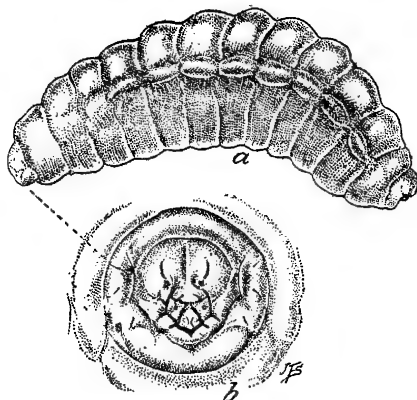


FIG. 10.—*Calliephialtes* sp.: a, Full-grown larva; b, face.

As a rule, the cocoon was started very shortly after the larva finished feeding, and for the purpose of this paper the beginning of the cocoon is taken as the end of the feeding period. However, in a considerable

number of cases some time elapsed after the larva had finished feeding before it began its cocoon, and in a few instances in which the insect was reared to maturity no cocoon was made. But such cases as that last mentioned resulted in diminutive adults. In most cases in which the cocoon making was delayed the supply of food had been small.

The feeding period—determined, as indicated above, from the hatching of the egg to the beginning of the cocoon—varied, in a total of 579 cases observed, from  $3\frac{1}{2}$  to  $18\frac{1}{2}$  days, with an average of about  $7\frac{1}{4}$  days. In Table II all of the larvæ carried through to the spinning of the cocoon are recorded, the months in which they spun their cocoons and their feeding periods being indicated. The weighted average feeding periods for each month and for the entire period are also shown. This is undoubtedly higher in each case than the normal average, because, while the conditions of nature were imitated so far as possible in the cages, abnormal influences affected some of the larvæ so that not only was their feeding period protracted, but some time passed after they had finished feeding before they started their cocoons. However, it is impossible to tell at what point to begin eliminating such larvæ from the averages, so all are included.

TABLE II.—*Actual and weighted average feeding periods of larvæ of Calliephialtes sp. for the period from May to October and the average for the season at Vienna Va., 1912.*

Feeding period.	Number of larvæ in—						
	May.	June.	July.	Aug.	Sept.	Oct.	Total.
3.5 days.....			3	2			5
4 days.....			14	7			21
4.5 days.....		1	19	8			28
5 days.....		7	45	37			89
5.5 days.....	7	32	20	12			71
6 days.....	28	16	21	7			72
6.5 days.....	18	26	13		1		58
7 days.....	14	16	14	2	2	1	49
7.5 days.....	7	14	2				23
8 days.....	8	12	2	2	3	7	34
8.5 days.....	5	8			1	1	15
9 days.....		2		2	3	3	10
9.5 days.....	1	4			1	5	11
10 days.....	5		1		2	10	18
10.5 days.....		1				9	10
11 days.....	1	1	2			17	21
11.5 days.....						4	4
12 days.....	1		1			9	11
12.5 days.....						1	1
13 days.....		1				4	5
13.5 days.....		1				2	3
14 days.....						5	5
14.5 days.....		1				2	3
15 days.....						4	4
15.5 days.....						4	4
16 days.....						2	2
16.5 days.....						1	1
18.5 days.....						1	1
Total.....	95	143	157	79	13	92	579
Average feeding period (days)...	6.98	6.85	5.54	5.21	8.42	11.53	7.07



A considerable portion of the larval life of *Calliephialtes* is passed in the cocoon. This period was determined for 116 female larvæ and 404 male larvæ. The females, after spinning their cocoons, required, on the average, about  $2\frac{1}{2}$  days longer to attain the pupal stage than did the males. This is probably somewhat less than the difference that would exist under natural conditions, inasmuch as the males under observation were somewhat more inclined to extend this portion of their development beyond the normal than were the females.

In Table III are brought together the data on that portion of the larval life passed within the cocoon. The figures include the prepupal period, which, not being a definite stage in the development of the insect but a transition stage, it is impossible to determine exactly. From this table are eliminated the data on 8 females and 11 males that remained in this condition for an abnormally long time. The actual maximum period recorded for females was 24 days and for males  $36\frac{1}{2}$  days.

TABLE III.—*Larval period of both sexes of Calliephialtes sp. in cocoon in various months, weighted average period for each month and for the season, and weighted average mean temperature for each period and for the season at Vienna, Va., 1912.*

Larval period in cocoon.	Females: Number of larvæ pupating in—				Total number of females.	Average mean temperature for period.	Males: Number of larvæ pupating in—				Total number of males.	Average mean temperature for period.
	May and June.	July.	Aug.	Sept.			May and June.	July.	Aug.	Sept.		
						° F.						° F.
4 days.....								I	I		2	78.0
4.5 days.....								2	I		3	78.2
5 days.....								II	I		12	76.5
5.5 days.....								I	9	2	12	74.8
6 days.....		I			I	77.8	II	34	24	I	70	74.2
6.5 days.....		I			I	77.7	13	19	13		45	72.7
7 days.....	I	I			2	72.3	20	23	20	4	67	71.0
7.5 days.....	I	3			4	74.4	17	8	II	I	37	72.4
8 days.....	8	3	I		12	72.2	23	10	14	9	56	71.7
8.5 days.....	4	3			7	73.9	22		5		27	67.7
9 days.....	II	I	3		15	70.9	10	2	10	2	24	69.6
9.5 days.....	7		2	2	11	69.6	7	I	I		9	68.5
10 days.....	8		2	I	11	68.4	5	I	2	3	11	71.1
10.5 days.....	6				6	67.5	I		2	I	4	70.9
11 days.....	16	I	I	4	22	68.8	2	3		2	7	72.4
11.5 days.....	4				4	69.0	3	I	I	2	7	72.1
12 days.....	4		I		5	69.1						
12.5 days.....												
13 days.....	4				4	67.7						
13.5 days.....	I				I	66.8						
14 days.....	2				2	67.6						
Total.....	77	14	10	7	108		135	125	108	25	393	
Weighted average period, days.....	10.2	8.0	9.7	10.4	9.9		7.9	6.6	7.2	8.7	7.4	
Average temperature, ° F.....						70.1						72.1

The figures of Table III are expressed in graphic form in the diagram (fig. 11), which shows the relation between temperature and the larval period in the cocoon. From the curve for males it is evident that individuals which took more than 8½ days between the spinning of the cocoon and pupation were more largely influenced by external conditions other than temperature than were those that required less time. The same is

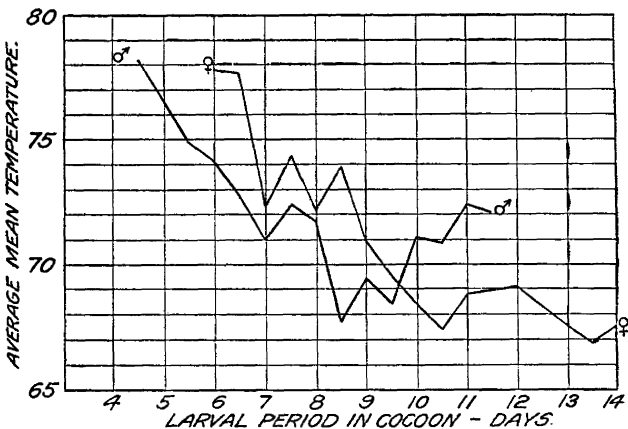


FIG. 11.—Diagram showing relation between temperature and larval period of males and females of *Calliephialtes* sp. in the cocoon at Vienna, Va., 1912.

true of the females after 10½ days, although these showed the effect to a less marked degree than did the males.

It will be seen from the figures given for the feeding period and the larval period in the cocoon that the minimum and maximum possible total larval periods

would be for females 9.5 and 42.5 days, respectively, and for males 7.5 and 55 days. The actual minimums and maximums were for females 12 and 27 days, respectively, and for males 7.5 and 51 days.

In Table IV are summarized the data obtained on the total larval period, with the exception of those on 13 females and 16 males in which this portion of the life cycle was unduly protracted. The total number for which the duration of this period was determined was 99 females and 344 males. The females required, in the average, nearly three days more to complete their larval life than did the males.

TABLE IV.—Summary of data on total larval period of *Calliephialtes* sp. at Vienna, Va., 1912.

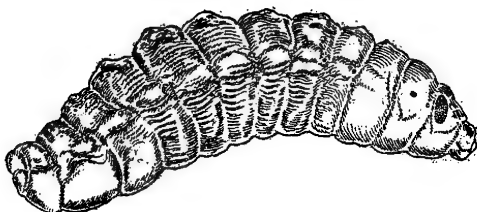
Total larval period.	Females: Number of larvæ pupating in—				Total number of females.	Males: Number pupating in—				Total number of males.
	May and June.	July.	August.	September.		May and June.	July.	August.	September.	
7.5 days.....							1			1
8.5 days.....								1		1
9 days.....								1		1
9.5 days.....							1	1		2
10 days.....							10	2		12
10.5 days.....							8	4		12
11 days.....						1	19	8		28
11.5 days.....						5	15	3	1	24

TABLE IV.—Summary of data on total larval period of *Calliephialtes*.; at Vienna, Va., 1912—Continued.

Total larval period.	Females: Number of larvae pupating in—				Total number of females.	Males: Number pupating in—				Total number of males.
	May and June.	July.	August.	September.		May and June.	July.	August.	September.	
12 days.....		2	1		3	10	25	15	4	54
12.5 days.....		3	1		4	12	4	4	1	21
13 days.....	1	3			4	9	7	16	2	34
13.5 days.....	2	1			3	18	3		2	23
14 days.....	2				2	11	5	8	1	25
14.5 days.....	5				5	18		1	1	20
15 days.....	7	1		2	10	10	2	5	1	18
15.5 days.....	6			1	7	5		2		7
16 days.....	4		1		5	13	1	4	2	20
16.5 days.....	10		1		11	6		1		7
17 days.....	3	1	1	2	7	2		2	1	5
17.5 days.....	9				9	1	2			3
18 days.....	6				6	3	1			4
18.5 days.....	2		1		3	2	1			3
19 days.....	3				3	1				1
19.5 days.....	4				4	1				1
20 days.....						1				1
Total.....	64	11	6	5	86	129	105	78	16	328
Average period, days.	16.5	13.3	15.4	15.9	16.0	14.3	11.9	12.8	13.6	13.2

## THE PREPUPA

A few days before pupation the larva begins to show the constriction between the thorax and the abdomen, the eyes become discernible as distinct red spots, and before pupation actually takes place the appendages can be indistinctly seen through the delicate larval skin. The antennæ are coiled under the head instead of being extended along the venter, as in the pupa. In the prepupal stage (fig. 12) the sex of the insect can with certainty be determined for the first time. In the female prepupa the tip of the abdomen is bent slightly backward, indicating the developing ovipositor, while in the male the caudal segment is straight.

FIG. 12.—*Calliephialtes* sp.: Prepupa of female.

## THE PUPA

When pupation takes place, the larval skin splits along the median dorsal line over the top of the head and for a short distance down the back, and through this opening the pupa makes its exit. Figure 13 shows the beginning of pupation of a female *Calliephialtes*. The rent in the exuvium, through which the antennæ are shown to extend, was

probably caused accidentally in the preparation of the specimen. By a series of twisting contortions the exuvium is gradually worked backward to the tip of the abdomen, where it is thrown off. It is very delicate and transparent, but as it is pushed back and becomes wrinkled it gradually appears darker until, when it is entirely shed, it is light grayish brown and is a mere shred.

In the male this is the end of the act of pupation, but it leaves the female with the ovipositor only a small fraction of its ultimate length and very thick.

The extension of the ovipositor is accompanied by a series of rythmical movements, about seven to the minute, during which the organ is repeatedly

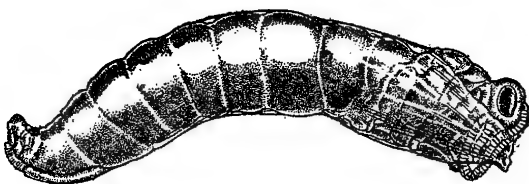


FIG. 13.—*Calliephialtes* sp.: Beginning of exuviation of female pupa.

pressed against the dorsum of the abdomen. Whether the pressure thus exerted is the cause of the lengthening of the ovipositor or the effect of pressure from within the body and merely incidental could not be determined.

The act of exuviation required about 15 minutes, but where the extension of the ovipositor was observed and timed the extension consumed from 35 to 41 minutes. The pupation of the male therefore required about 15 minutes, while the female required from 50 to 56 minutes to complete the process.

The newly formed pupa is entirely white, with the exception of the eyes, which are red. The legs and antennæ lie fully extended along the sides and venter, and in the female the ovipositor lies along the dorsum, extending the whole length of the body and curving somewhat at its tip over the head.

Gradually the eyes darken, becoming very dark before the adult color begins to appear over the rest of the body. The head and thorax are the next to begin to assume color, then the dorsal and ventral plates of the abdomen, the antennæ, the legs, and finally the ovipositor. When the coloring is complete (see fig. 14), the head, thorax, and antennæ are black, the eyes dark reddish brown, the wing pads gray, the chitinized portions of the abdomen and ovipositor nearly black, the legs yellowish, and the unchitinized portions white.

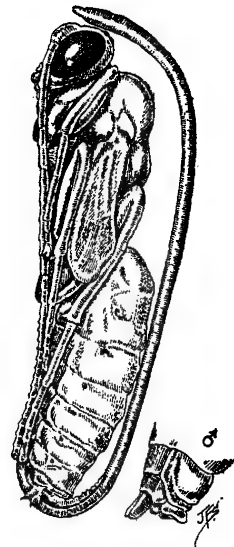


FIG. 14.—*Calliephialtes* sp.: Pupa of female and tip of abdomen of male pupa.

The pupal periods of 109 females and 366 males were determined. The average female spent 1.66 days longer in this stage than did the average male. This difference would, however, probably be somewhat greater

under natural conditions, as the males under observation were considerably more likely to extend this period beyond the normal than were the females. The actual difference is probably more closely indicated by the shortest pupal period for each sex, which gives a difference of two days.

In Table V the data on the pupal period are summarized and the average mean temperature for the various periods given.

TABLE V.—Summary of data on duration of pupal period of *Calliephialtes* sp. and average mean temperature at Vienna, Va., 1912.

Pupal period.	Females: Number transforming in—				Total number of females.	Average mean temperature.	Males: Number transforming in—				Total number of males.	Average mean temperature.
	June.	July.	Aug.	Sept.			June.	July.	Aug.	Sept.		
						°F.						°F.
6 days.....								1	1		2	77.2
6.5 days.....								1			1	79.0
7 days.....								17	8	3	28	78.2
7.5 days.....							1	2	5	9	17	74.7
8 days.....		2	1		3	78.7	2	11	9	23	45	74.4
8.5 days.....		1	1		2	72.4	4	8	11	14	37	73.4
9 days.....		2	2	5	9	76.9	17	9	14	21	61	68.4
9.5 days.....		3		1	4	72.6	22		14	1	37	68.3
10 days.....	5	10		3	18	71.9	51		28	2	81	68.0
10.5 days.....	7	6	1	4	18	71.6	18		16		34	65.3
11 days.....	10	1	2		13	69.9	19		1		20	66.9
11.5 days.....	17				17	68.6	3				3	66.4
12 days.....	15				15	68.9						
12.5 days.....	5	1			6	67.5						
13 days.....	4				4	66.7						
Total.....	63	26	7	13	109	.....	137	49	107	73	366	.....
Average pupal period, days.....	11.50	9.90	9.57	9.73	10.78	.....	9.94	7.83	9.19	8.36	9.12	.....
Average temperature, °F.....						70.9						70.3

The August column for males in Table V includes the data on 49 pupæ which were reared from unfertilized eggs. Whether the parthenogenetic character of these eggs had any effect in lengthening the pupal period is a question, but a comparison of the pupal periods of these with those of the 34 males that were developing at the same time from fertilized eggs shows that the pupæ from parthenogenetic eggs required a somewhat longer time. This is shown in Table VI. If these 49 individuals were eliminated in Table V, the total and average in the August column would be 58 and 8.61, respectively, and the grand total and grand average would be 317 and 9.0, respectively.

TABLE VI.—Relative length of pupal stage of males of *Calliephialtes* sp. from fertilized eggs and those from parthenogenetic eggs at Vienna, Va., 1912.

Pupal period.	Number of pupæ from—	
	Fertilized eggs.	Parthenogenetic eggs.
7.5 days.....	1	.....
8 days.....	1	.....
8.5 days.....	3	3
9 days.....	8	6
9.5 days.....	9	6
10 days.....	7	21
10.5 days.....	5	12
11 days.....	.....	1
Total.....	34	49
Average pupal period, days.....	9.44	9.87

The truth of the relation between the pupal period and temperature is in all probability not nearly so closely shown by the figures

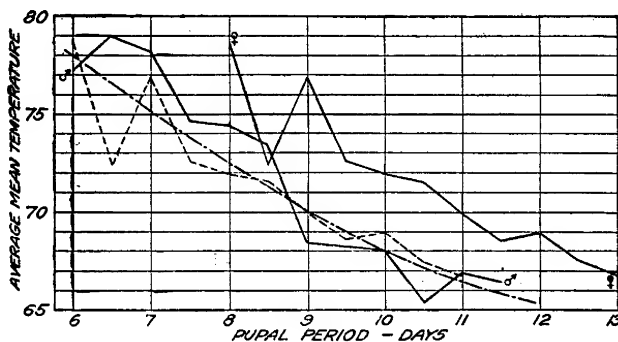


FIG. 15.—Diagram showing relation between pupal period of *Calliephialtes* sp. and temperature. The dot-and-dash line is the curve of average temperature, while the dotted line represents the female curve superimposed on that of the males at Vienna, Va., 1912. The greater tendency of the males to delay transformation to the adult stage is shown by referring the male and female to the line of average temperatures.

as is that between the incubation period and temperature by the figures in Table I, since the important factor of amount and condition of food has had opportunity to have its full effect. This factor, next to temperature, is probably the most important single factor influencing the duration of the stage, especially

under the unnatural condition of the breeding cage.

The figures of Table V are expressed in graphic form in figure 15.

#### THE COCOON

As stated, the larva usually begins its cocoon shortly after having finished feeding. Before starting its spinning it pushes the remains of its host to one end of the host cocoon and then accommodates its own cocoon to the size and shape of the space remaining within that of the host. The parasite cocoon therefore varies considerably in shape. It is usually, however, about one-half inch in length, about a third as broad,

and of the depth of the host cocoon. The upper and lower sides and the end next the remains of the host, most frequently the cephalic end, are flattened, while the edges and the other end are more rounded.

The cocoon is of a pale pinkish brown color, semitransparent, and is composed of a thin tissuelike material containing but few threads.

It was found very easy to observe by transmitted light the development of the parasite in its cocoon.

The period in the cocoon includes a part of the larval life, all of the pupal period, and a small portion of the adult life. By adding the minimums and maximums for each of these phases of development the total possible minimum period would be for females 14.5 days and for males 10.5 days and the total possible maximum period for females 39 days and for males 50 days. The actual minimum and maximum for females were 15.5 and 37.5 days, respectively, and for males 11.5 and 36 days.

The duration of this period was determined for 111 females and 396 males. The weighted average duration of this portion of the life history indicates that the females remain in the cocoon about four days longer than do the males. Table VII summarizes the data obtained. Eight females and five males, the recorded periods of which were far in excess of the normal for the month in which they emerged, are omitted from the table.

TABLE VII.—Summary of period spent by *Calliephialtes* sp. in cocoon, showing number for each period by months, total for each period and each month, and average period for each month and for the season at Vienna, Va., 1912.

Period in cocoon.	Number of females emerging in—				Total number of females.	Number of males emerging in—				Total number of males.
	June.	July.	Aug.	Sept.		June.	July.	Aug.	Sept.	
11.5 days. . . . .								1		1
12 days. . . . .										
12.5 days. . . . .							1			1
13 days. . . . .							10	1		11
13.5 days. . . . .							4	1		5
14 days. . . . .							4			4
14.5 days. . . . .							2	1		3
15 days. . . . .							9	6	6	21
15.5 days. . . . .		1			1		3	6	5	14
16 days. . . . .		1			1	2	5	16	15	38
16.5 days. . . . .		1			1			11	11	22
17 days. . . . .		2			2	8	3	29	18	58
17.5 days. . . . .						13	3	20	7	43
18 days. . . . .		2			2	26	2	17	5	50
18.5 days. . . . .			1		1	19	1	4	2	26
19 days. . . . .		2	1	1	4	20		7	3	30
19.5 days. . . . .		3	1	4	8	12	1	5		18
20 days. . . . .	2	2	3	1	8	11	1	2	3	17
20.5 days. . . . .	2	4		1	7	4	1	1	1	7
21 days. . . . .	6	1		4	11	8		1	1	10

TABLE VII.—Summary of period spent by *Calliephialtes* sp. in cocoon, showing number for each period by months, total for each period and each month, and average period for each month and for the season at Vienna, Va., 1912—Continued.

Period in cocoon.	Number of females emerging in—				Total number of females.	Number of males emerging in—				Total number of males.
	June.	July.	Aug.	Sept.		June.	July.	Aug.	Sept.	
21.5 days. . . . .	8	1	.....	.....	9	2	.....	.....	.....	2
22 days. . . . .	13	1	.....	1	15	1	.....	1	1	3
22.5 days. . . . .	4	1	.....	.....	5	1	.....	.....	.....	1
23 days. . . . .	11	1	1	1	14	2	.....	.....	.....	2
23.5 days. . . . .	3	.....	.....	.....	3	1	.....	.....	.....	1
24 days. . . . .	1	.....	.....	.....	1	1	.....	.....	.....	1
24.5 days. . . . .	3	.....	.....	.....	3	2	.....	.....	.....	2
25 days. . . . .	3	.....	.....	.....	3	.....	.....	.....	.....	.....
26 days. . . . .	2	.....	.....	.....	2	.....	.....	.....	.....	.....
26.5 days. . . . .	1	.....	.....	.....	1	.....	.....	.....	.....	.....
27 days. . . . .	1	.....	.....	.....	1	.....	.....	.....	.....	.....
Total number. . . . .	60	23	7	13	103	133	50	130	78	391
Average period, days. .	22.6	19.4	20.0	20.5	21.5	19.4	15.3	17.2	17.1	17.7

#### THE ADULT

Transformation from the pupa to the adult within the cocoon takes place one or two days before the emergence of the adult, depending largely on the difficulty encountered by the insect in biting its way by the remains of the host and through the two cocoons. The female effects her escape in a somewhat shorter time than the male.

In the spring the males appear some time ahead of the females, as indicated by the emergence of unforced material in the spring of 1912. From this material the first males appeared on April 23 and the first females 10 days later. In fact all but a few belated males appeared before the first female.

The males far outnumbered the females throughout the period covered by the observations, and it was found that the proportion of males increased with each succeeding brood. It appears that the effect of the unavoidably unnatural conditions of the artificial propagation tended to the production of males and that this effect was cumulative. Of the 528 individuals reared from mated females in the regular life-history experiments 396, or exactly three-fourths, were males. Table VIII summarizes the data on this point.



TABLE VIII.—*Proportion of sexes of Calliephialtes sp. from bisexual reproduction at Vienna, Va., 1912.*

Brood.	Number of females.	Number of males.	Ratio of females to males.
Hibernating.....	21	52	1:2.48
First.....	82	153	1:1.87
Second.....	20	112	1:5.60
Third.....	9	79	1:8.79
Total.....	132	396	1:3.00

Of the 57 individuals reared from parthenogenetic eggs all were males.

No definite data were obtained on the longevity of the females, for the reason that it was necessary to use all such in propagation experiments, and the individuals could not be distinguished. Some information on this point can, however, be obtained from the notes on the propagation cages. All females were fed, and hence there are no data on longevity without food.

Of the unforced hibernating females the first emerged on May 3 and the last on May 13. The latter was a weak individual and lived only 10 days. The last to emerge previous to it appeared on May 7. The earliest death, with the exception mentioned above, occurred on June 4 and the last on June 22. This gives a maximum longevity of 50 days, a minimum of 22 days, and an average of 36 days.

Females emerging from June 13 to 17 died from July 9 to August 7. The maximum longevity was 55 days, the minimum 22 days, and average 38.5 days.

Females emerging from June 24 to 26 died from July 19 to August 9. The maximum longevity was 46 days, the minimum 23 days, and the average 34.5.

Females emerging from June 27 to July 1 died from July 9 to 30. The maximum longevity was 33 days, the minimum 8 days, and the average 20.5 days.

The females surviving on August 9 in all first-generation cages were assembled in one cage on that date. Of these, 4 were from a lot emerging from June 18 to 20, an average of 51 days previous to the transfer; 3 from a lot emerging from June 22 to 23, an average of 47.5 days previously; 3 emerging from July 3 to 10, 33.5 days previously. The 10 females, after being placed together, died August 13 to 19, an average of 7 days later. The average longevity of the females from the earliest of the three lots was therefore 58 days, of those from the second lot 54.5 days, and of those from the third lot 40.5 days.

The average longevity of all females listed above was 51 days.

A number of surplus males emerging from June 14 to 22 were used in an experiment to determine the longevity with and without food. Of the 51 males used in the experiment 22 were fed and 29 unfed. For the fed males the maximum longevity was 51.5 days, the minimum 8.5 days, and the weighted average 32.5 days. The longest lived unfed male lived 10 days, the shortest lived 3 days, and the average lived 5.4 days. The average fed male therefore lived almost exactly six times as long as the average unfed male.

The adult *Calliephialtes* were very easily handled on account of their great docility. On many occasions while photographing the females in the act of oviposition the writer has carried a transparent slide on which a female was perched from the insectary to a greenhouse 20 feet distant, set it up in front of the camera, and made one or more exposures without the insect withdrawing her ovipositor; and in no case was the insect sufficiently disturbed to cause her to fly away.

The adults fed greedily at all times on the sweet liquids supplied them, and the males confined their feeding to this sort of diet. But the females very frequently fed on the juices of the codling-moth larvæ. This food they secured by repeatedly jabbing with their ovipositors the larvæ in the cocoons and licking up the juices that saturated the cocoon. Frequently a half or more of a larva would be consumed in this way, the parasite continuing to feed for an hour or more, alternately pumping the juices of the larvæ out with her ovipositor and licking them up. On one occasion a female *Calliephialtes* was observed to have killed and partially eaten a larva that had left its cocoon and was at large in the cage.

The total developmental period from oviposition to emergence was determined for 112 females and 399 males. For females it ranged from 23.5 days to 44.5 days and for males from 18 to 44 days. Both of the maximums as well as a considerable number of other records are based on individuals which, for some cause—usually inadequate food supply—were unable to go through their development in as short a time as they would have done under normal conditions. The records for 12 such females and 22 males are omitted from Table IX, which summarizes the data on the 100 other females and 377 other males. This table indicates that the average female required about 5 days longer to complete development than did the average male, the shortest period for females being  $5\frac{1}{2}$  days longer than the shortest for males.

TABLE IX.—*Total developmental period of Calliephialtes sp.; summary of duration of period by months, sexes, and for the season at Vienna, Va., 1912.*

Total develop- mental period.	Number of females emerging in—				Total number of fe- males.	Number of males emerging in—				Total number of males.
	June.	July.	Aug.	Sept.		June.	July.	Aug.	Sept.	
<i>Days.</i>										
18.....							1			1
18.5.....							1			1
19.....								1		1
19.5.....							2	1		3
20.....							2	1		3
20.5.....							9	1		10
21.....							11	4	2	17
21.5.....							3	3	4	10
22.....							5	5	5	15
22.5.....							2	10	11	23
23.....							3	14	14	31
23.5.....		3			3		1	18	13	32
24.....		2			2	2	3	19	8	32
24.5.....							1	9	4	14
25.....		2	1	1	4	10		14	3	27
25.5.....						10		7	4	21
26.....		2	3	2	7	14		6		20
26.5.....		1		2	3	17	1	3	3	24
27.....		1	1	2	4	21	1	4	2	28
27.5.....	1			2	3	8	1	6		15
28.....				2	2	17		4	1	22
28.5.....	3	2			5	2				2
29.....	8	2			10	15				15
29.5.....	6		1	2	9	1				1
30.....	8	2	1		11	4				4
30.5.....	6				6					
31.....	6	1			7	2				2
31.5.....	2	1			3	3				3
32.....	2	2			4					
32.5.....	3				3					
33.....	6				6					
33.5.....	1				1					
34.....	1				1					
35.....	1				1					
35.5.....	1				1					
36.....	4				4					
Total....	59	21	7	13	100	126	47	130	74	377
Average de- velopmen- tal period, days.....	31.1	27.4	27.1	27.2	29.6	27.2	21.7	24.1	23.5	24.7

No definite experiments were conducted in experimental control of the development, but during the warm weather many strawboard slips of parasitized larvæ were placed in cold storage to retard the development of the parasites. In so far as it was possible to determine, they were placed in storage after the spinning of the parasite cocoon. This retardation of development had no apparent effect on the further development after removal from cold storage. It did seem, however, to reduce the activity and vitality of the resulting adults. L. J. Newman (18) records

the keeping of immature specimens of *Calliephialtes messor* in cold storage for a period of 14 months, after which they emerged without having suffered in the least.

#### SEASONAL HISTORY

The first females to emerge from hibernation in the spring of 1912 appeared on May 3 and the last on May 15. These were placed with males in propagation cages. The first egg was deposited on May 13, ten days after the first emergence.

In order to determine the maximum and minimum number of generations in a season, the five earliest and five latest appearing female progeny of the hibernating brood were used in the life-history cages, a separate cage being used for each group. The same plan was followed out with each succeeding generation. From the earliest female progeny three complete generations were reared, and from the latest group two generations were bred. With the hibernating brood this gives a maximum of four generations in the year and a minimum of three generations. Table X summarizes the data on the number of generations. It is interesting to note that the total time consumed by the three generations is only one day longer than that consumed by the two.

TABLE X.—Number of generations of *Calliephialtes* sp. reared at Vienna, Va., 1912.

Generation.	Maximum number of generations.		Minimum number of generations.	
	Date of first female.	Total cycle.	Date of last female.	Total cycle.
Hibernating.....	May 3	Days.....	May 13	Days.....
First.....	June 13	41	July 13	61
Second.....	July 18	35	Sept. 12	61
Third.....	Sept. 3	47	.....	.....
Total period, days.....	.....	123	.....	122
Average cycle, days.....	.....	41	.....	61

Development ceased at about 50° F., although oviposition was frequently carried on actively at that temperature. After the middle of October very few eggs hatched, although the last eggs of the season were not deposited until November 1. All but a very few of the larvæ that hatched at this season passed through the feeding stage and constructed their cocoons.

*Calliephialtes* sp. hibernates as a full-grown larva in its cocoon. In this stage it is capable of withstanding a very low temperature. The mortality among hibernating larvæ during the winter of 1911-12 was very slight, if not nil, in spite of the fact that a temperature of -6°

Fahrenheit was recorded in the insectary. This is an unusually low record for the locality and indicates that the species would have no difficulty in acclimating itself were it liberated in the region.

#### ALTERNATE HOSTS

The female parasites appeared in the spring a few days in advance of the first adult codling moth, or somewhere about 40 days before they could, under natural conditions, attack the first brood of larvæ of the codling moth. The hibernating brood of parasites would therefore have passed the greater portion of their adult life before an abundance of codling-moth larvæ could be found. This would necessitate a very small first generation of the parasites unless they would attack some other host.

To determine if *Calliephialtes* would attack other species of insects, larvæ of *Enarmonia prunivora* Walsh, *Euzophera semifuneralis* Walk., and *Gnorimoschema gallaesolidaginis* (Riley) were placed in the propagating cages with actively ovipositing female parasites. The larvæ of the first two species were placed in transparent cells, and those of the last were allowed to remain in their galls. Only a single *Enarmonia* larva was available, and this was parasitized within 2 days, a diminutive male *Calliephialtes* emerging from the cocoon 22 days later. This species is, however, much smaller than the normal full-grown larva of the parasite, and it is doubtful if it would serve in the long run as an alternate host.

Of the two other species of larvæ neither was apparently given the least attention by the parasites, although those of *Euzophera* were left in the cage for several weeks.

Codling-moth larvæ containing the internally parasitic larvæ of *Ascogaster carpocapsae* were readily attacked and parasitized by *Calliephialtes*. This always resulted in the death of the earlier parasite and the production of a diminutive adult *Calliephialtes*.

On one occasion a *Calliephialtes* larva that had already spun its cocoon was attacked and killed by an adult of the same species. When the fact was discovered, a small living larva was feeding on the dead parasite larva. This parasite larva died without spinning.

#### LITERATURE CITED

1. GRAVENHORST, J. L. C. *Ichneumonologia Europæa*. v. 3, Vratislaviae, 1829.  
"Ephialtes messor, n.," p. 232. Original description.
2. TASCHENBERG, E. L. Die Schlupfwespenfamilie Pimplariæ der deutschen Fauna, mit besonderer Rücksicht auf die Umgegend von Halle. *Ztschr. Ges. Naturw.*, Bd. 21, p. 245-305.  
"Ephialtes messor Gr.," p. 254. Included in synoptic table of genus and recorded as reared from the wax moth. (*Tinea*) *Galleria mellonella*.
3. WALSH, B. D. Descriptions of North American Hymenoptera. *Trans. Acad. Sci. St. Louis*, v. 3, p. 65-166, 1873.  
"Ephialtes pusio, n. sp.," p. 111-112. Original description.

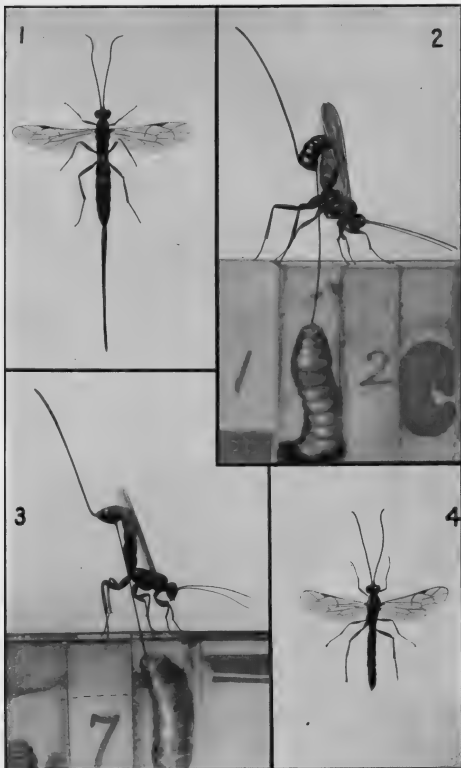
4. CRESSON, E. T. "*Ephialtes comstockii* Cresson (n. sp.)." U. S. Comr. Agr. Rpt., 1879, p. 235, 1880.  
Original description. Type reared as parasite of *Retinia comstockiana* Fernald.
5. DALLA TORRE, K. W. VON. Catalogus Hymenopterorum. v. 3, Lipsiae, 1901-2.  
"*Ephialtes messor* Grav.," p. 475; "*Ephialtes comstocki* Cress.," p. 471; "*Ephialtes pusio* Walsh.," p. 476. Credits Gravenhorst with having recorded *Tinea mellonella* as host of *E. messor* Grav. This record should be accredited to Taschenberg (1863).
6. COOPER, ELWOOD. The codling-moth parasite. 2d Bien. Rpt. Comr. Hort. Cal., 1905/6. p. 231-235, pl. 10. 1907.  
"The codling-moth parasite (*Caliephialtes messor* Grav.)," p. 231-235, pl. 10. Short general account of introduction into California, together with description, life history, habits, and letters from fruit growers regarding success of introduction.
7. LOUNSBURY, C. P. Report of the Government Entomologist [Cape of Good Hope], 1905. 1906.  
"*Ephialtes messor* Gravenhorst," p. 98-99. Mentions introduction into California and expresses doubt as to probable success.
8. FROGGATT, W. W. Codling-moth parasites. Agr. Gaz. N. S. Wales, v. 17, pt. 4, p. 387-395, Apr. 2, 1906.  
"The Spanish parasite (*Ephialtes carbonarius*)," p. 393-394. Mentioned in list of codling-moth parasites.
9. LOUNSBURY, C. P. Report of the Government Entomologist [Cape of Good Hope], 1906. 1907.  
(Spanish parasite), p. 86. Mentions introduction into California late in 1904. Doubts value.
10. ——— Report of the Government Entomologist [Cape of Good Hope], 1907. 1908.  
"*Caliephialtes messor*," p. 55. Records introduction into Cape of Good Hope from California. Comments on introduction into California and expresses belief that as yet the species has not proved of any practical value or given evidence that it will.
11. SCHREINER, J. T. Zwei neue interessante Parasiten der Apfelmade *Carpocapsa pomonella* L. Ztschr. Wiss. Insektenbiol, Bd. 3, Heft 7, p. 217-220, 1 fig., Dez. 9, 1907.  
"*Ephialtes carbonarius* Christ.," p. 218. Records rearing from codling-moth larva in Europe.
12. QUAINANCE, A. L. The codling moth or apple worm. U. S. Dept. Agr. Year-book, 1907, p. 432-450, 1908.  
"*Calliephialtes messor* Grav.," p. 443. Mentioned in list of parasites of codling moth as having been introduced into California to prey upon that insect.
13. FROGGATT, W. W. Insect pests in foreign lands. Second progress report. Jour. Dept. Agr. Victoria, v. 5, pt. 12, p. 716-720.  
"*Ephialtes carbonarius*," p. 717. States that in visit to California he was unable to find any instance in which the parasite had been found in any orchard.
14. LOUNSBURY, C. P. Report of the Government Entomologist [Cape of Good Hope], 1908. 1909.  
"Spanish codling-moth parasite. (*Caliephialtes messor*)," p. 64. Records experience in rearing parasite.
15. THEOBALD, F. V. The insect and other allied pests of orchard, bush, and hothouse fruits and their prevention and treatment. Wye, 1909.  
"The codling moth ichneumon (*Ephialtes carbonarius* Zach.)," p. 77-78. Mentions introduction into California. Brief life history.
16. FROGGATT, W. W. Report on Parasitic and Injurious Insects, 1907-8. Sydney, 1909.  
"*Calliephialtes messor*," p. 5-7. Doubts efficiency of species in California.

17. LOUNSBURY, C. P. Report of the Government Entomologist [Cape of Good Hope], 1909. 1910.  
    "*Calliephialtes messer*," p. 85-86. Reports further liberations. No hope held out that species will prove of importance.
18. NEWMAN, L. J. Long-lived parasites. Jour. Dept. Agr. West Aust., v. 18, pt. 4, p. 297, Apr., 1909.  
    "*Calliephialtes messer*." Records keeping moth larvæ parasitized by this species in cold storage 14 months, after which the parasites emerged, apparently not having suffered from the long cold.
19. ESSIG, E. O. Injurious and beneficial insects of California. Mo. Bul. Cal. State Com. Hort., v. 2, no. 1-2, Jan./Feb., 1913.  
    Brief description and biologic remarks on *Calliephialtes messer* Grav., p. 265-266, fig. 264.

#### DESCRIPTION OF PLATE

PLATE XX. *Calliephialtes* sp. Fig. 1.—Female. Figs. 2 and 3.—Characteristic positions assumed by the insect in oviposition. Fig. 4.—Male. Figures 1 and 4 are enlarged about  $2\frac{1}{2}$  times. Figures 2 and 3 are retouched photographs from life; enlarged about 3 times.





## POLYPORUS DRYADEUS, A ROOT PARASITE ON THE OAK

By W. H. LONG,

*Forest Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry*

Bulliard (1789, 1791)<sup>1</sup> figured and described under the name *Boletus pseudo-igniarius* a fungus which most European mycologists believe is the plant now called *Polyporus dryadeus*. Apparently the next record of this fungus is by Persoon (1799), where it is described as *Boletus dryadeus*. Again it is described by the same writer in his *Synopsis Fungorum* (1801), where Bulliard's fungus is listed as a synonym. It is first named *Polyporus dryadeus* by Fries (1821), who describes the plant and cites as synonyms the names given by Bulliard and Persoon. Hussey in *Illustrations of British Mycology* (1849) gives a fairly good figure of the sporophore and a most excellent mycological description of the fungus, with its habitat.

Since 1849, repeated references to this fungus are found in European mycological literature, but nothing was written concerning the rot produced by it in the oak until Robert Hartig in his epoch-making work on the true nature of the rots of woods (1878) described a heart rot of the oak which he attributed to *Polyporus dryadeus*. A careful study of Hartig's figures and the description of the sporophore which he found associated with the white heart-rot so accurately described by him is sufficient to convince anyone who is familiar with the true *P. dryadeus* that Hartig's fungus was not *P. dryadeus*. It is undoubtedly identical with the heart-rotting fungus known in America as *P. dryophilus* and found by Hedgcock (1910 and 1912) to be associated with a whitish piped rot in oak.

*Polyporus dryophilus* has one character, a hard, granular, sandstone-like core, that is unique and not possessed by any other polypore known to the writer. The sporophore of this plant, represented by numerous specimens collected by Hedgcock and the writer in western and southwestern United States, shows this hard, granular core exactly as figured and described by Hartig in his article on *P. dryadeus*. This core extends back some distance into the tree in oaks; it is usually irregularly cylindrical while in the tree, but on its emergence from the tree it swells into a tuberous or spheroid mass and finally occupies the central and rear part of the sporophore. (Pl. XXI, fig. 1.) If the sporophore is formed from a large branch hole, it is usually of the applanate type, with a small core, but when the sporophore forms directly on the body of the

<sup>1</sup> Bibliographic citations in parentheses in the text of this article refer to "Literature cited," p. 248.

tree, as it usually does, the shape is tuberous, ungulate, or even subglobular (Pl. XXI, figs. 2 and 3), with the bulk of the sporophore composed of a hard, granular core. This core usually has white mycelial strands. (Pl. XXI, fig. 3.) The sporophore of *P. dryophilus*, therefore, has normally three distinct kinds of structures: (1) The hard, granular core, (2) the fibrous layer which surrounds this core except at the rear, and (3) the layer of tubes on the lower surface. Specimens are often found, however, especially from the western part of the United States, in which this fibrous layer may be entirely absent between the tubes and the granular core. (Pl. XXI, fig. 3.)

The sporophore of *Polyporus dryadeus* never has this granular core, but its context is fairly homogeneous and of a fibrous-corky structure. (Pl. XXI, fig. 4.) Another very important difference between the two species is the location of the sporophores on the host tree. In *P. dryadeus* the sporophores are always on the exposed roots or on the trunks at or very close to the ground. The reason for this is explained later in this article. In *P. dryophilus* the sporophores are higher on the trunk of the tree, and in some cases are on the branches.

The rot described and figured by Hartig is identical with the rot produced by *P. dryophilus*, but does not resemble in the least the rot produced by the real *P. dryadeus*. Since Hartig's time European mycologists have followed him in descriptions of the rot wrongly ascribed to *P. dryadeus*, but most of them have described the sporophores of the true *P. dryadeus* both as to its character and location on the tree—i. e., at the base of oaks. For instance, Von Tubeuf, in his *Disease of Plants* (1897), describes fairly well the sporophore of *P. dryadeus*, while his photograph of the rot is that of *P. dryophilus*. Massee, in his *Diseases of Cultivated Plants and Trees* (1910), states that "the largest specimens usually occur near the ground line, but it also springs from points where branches have died or been broken off." The latter statement, so far as can be ascertained by the writer, is incorrect as to the location of the sporophores of *P. dryadeus*, but is correct for *P. dryophilus*. Massee also quotes Hartig as to the character of the rot produced.

*Polyporus dryophilus* is known in Europe under at least three different names: *Polyporus fulvus* Fries (Pl. XXI, fig. 5), *P. friesii* Bresadola, and *P. vulpinus* Fries. (Pl. XXI, fig. 6.) According to Lloyd (1913), not only are *P. fulvus* Fries and *P. friesii* Bresadola synonyms for *P. dryophilus*, but the *P. corruscans* of Fries is also the same plant.<sup>1</sup> *Polyporus vulpinus* is the name given to the form of *P. dryophilus* found on species of *Populus*, authentic specimens of which were seen by the writer at the New York Botanical Garden in collections from Finland and Sweden and also from

<sup>1</sup> Since this article was written, the writer, through the courtesy of Mr. C. G. Lloyd, has examined the specimens of *Polyporus corruscans* and of *P. rheades* deposited in the Lloyd Herbarium at Cincinnati, Ohio. Both of these plants as represented in this herbarium are *Polyporus dryophilus*, the former being the usual form found on oak and the latter the one occurring on poplar. According to Mr. Lloyd, the type of *P. rheades*, found by him in Persoon's Herbarium, is undoubtedly the plant called "*P. vulpinus*" by Fries.

Maine. In the Cryptogamic Herbarium of Harvard University there is a collection on *Populus grandidentata* Michx. from New Hampshire, while in the laboratory of forest pathology of the Department of Agriculture at Washington, D. C., there is a fine collection on *Populus tremuloides* Michx. from near Steamboat Springs, Colo. (Hedgcock, 1913).

This fungus on *Populus* agrees in all essential characters with the form of *Polyporus dryophilus* found on oak. The sporophores are, however, somewhat smaller than those usually found on oak and approach the applanate type. (Pl. XXI, fig. 7.) The hard granular core is always present, but is formed between the sapwood and bark (Pl. XXI, fig. 8), as the fungus is able to rot the sapwood as well as the heart of this host. It therefore does not have to depend on branch holes or other openings through the sapwood in order to form its sporophores, as it does in the oak.

Through the kindness of Von Tubeuf the writer obtained a European specimen of Hartig's so-called rot of *Polyporus dryadeus* in oaks. (Pl. XXII, fig. 1.)<sup>1</sup> It is unquestionably the rot produced by *P. dryophilus*. (Pl. XXII, fig. 3.)

The following discussion of the rot caused by *Polyporus dryadeus* embodies the results obtained from extensive field studies made in the forests of Arkansas, eastern Texas, Oklahoma, Maryland, and Virginia.

The sporophores of *P. dryadeus* are always found at or very near the ground at the base of the host. This first suggested to the writer that the fungus might be a true root-rotting organism. Trees with sporophores at their bases and wind-thrown oaks with and without the sporophores attached were carefully studied. Sections of the trees were cut, roots dug up and examined, and every effort made to determine exactly the character of the rot produced. The roots and stools of 20 trees attacked by this disease were examined, and sections of the various stages of the rot were studied.

The microscopic characters of the rot from each tree were found to be identical, although of the 20 trees examined 5 were in Arkansas, 3 in Texas, 2 in Oklahoma, 4 in Maryland, and 6 in Virginia. In every instance the trees were found to have a white rot which attacks first the sap and finally the heartwood of the roots. The rot originates in the lower portion of the roots and spreads in them toward the base of the tree.

The first evidence of the disease is a reddish brown discoloration of the inner bark and cambium. If the diseased roots are exposed in a damp chamber at this stage, white floccose spots of mycelium will appear on the outside of the bark, but the rot has not yet become evident in the wood. As the rot progresses, discolored, watery, reddish

<sup>1</sup> Figure 1 on Plate XXII was made from a photograph of a piece of the original type material used by Hartig in his description of the rot of *Polyporus dryadeus* (1878).

brown areas, which become hazel in color when the wood is dried, appear on the surface of the sapwood and in its outer layers. At this stage a cross section of the root has a mottled appearance, and this discoloration gradually spreads till the root is affected to its center. The earliest discolored spots have by this time turned white. (Pl. XXII, fig. 2.) Later, as the rot ages, especially in the larger roots which lie near the surface of the ground, this white changes to a cream and finally to a straw color. The lower portion of the smaller diseased roots, those 2 inches or less in diameter, become completely rotted and white throughout before the advancing rot has reached the stool of the tree. On these small rotted roots the bark separates easily from the wood, since much of the living bark has been destroyed. The bast fibers, however, remain intact, which gives the inner bark a loose, shredded appearance. The rot gradually moves up the roots till the stool is reached. This is also attacked by the fungus, but the rotted area ends abruptly at the surface of the ground.

A radial-longitudinal section of the rot in a fresh state has a sodden, watery appearance, with white longitudinal and transverse lines somewhat like the rot produced by *Polyporus hispidus* in oaks. These white lines or bands are not cellulose, however, but are spaces filled with air and the mycelium of the fungus in the region of the large vessels. When the rotted wood is thoroughly dry, these white lines disappear, and the uniformly creamy-white rot is left. The rot in all the trees examined did not extend for any appreciable distance into the heartwood of the trunk proper above the collar of the tree, even when the large, completely buried roots, 6 to 12 inches in diameter, were rotted throughout.

The thoroughly rotted wood when dry is very light in weight and, superficially, looks and feels like pith. If a freshly dug root in the advanced stage of the rot is twisted, it will split into concentric layers and also into longitudinal blocks, giving the broken end of the root a coarse, fibrous appearance. The lower ends of the diseased roots may be in a thoroughly rotted condition, easily splitting into these concentric layers and rough, fibrous masses, while that portion of the root next to the base of the tree remains comparatively sound. The roots of several of the trees overthrown by the wind were thus affected. The presence of this rot is often indicated by irregular white mycelial patches on the outside of the bark of the root or of the stool of the tree.

In a radial-longitudinal section through the heartwood of a diseased root the advancing line of the rot first appears as a watery dark-brown zone 1 to 3 inches wide. This dark area terminates rather abruptly in the ultimate cream-colored rot on one side and in the sound heartwood on the other. A microscopic examination of the diseased wood shows that the starch and other cell contents of the roots are first extracted; then the walls of the wood elements are gradually destroyed, especially the walls of the tracheids and vessels adjacent to the large medullary

rays. The bordered pits in the vessels are usually reduced to long, elliptical openings running transversely across the walls, and the bordered pits of the tracheids become large, round holes, which often coalesce, thus splitting the tracheids longitudinally. The pits of both large and small medullary rays are somewhat enlarged, while their radial and tangential walls are perforated with holes.

Even in the early stages of the rot, when the discolored spots are beginning to show in the sapwood of the roots, the vessels have colorless hyphæ in them, while in the later stages many of the vessels become filled with a mass of colorless hyphæ having filaments  $4\mu$  or less in diameter. The wood-parenchyma fibers show enlarged pits and perforated radial walls, and the pits in the wood fibers are also enlarged. The walls of the medullary rays are much corroded and often disappear entirely.

Only very slight evidence of delignification is shown by the chloriodid of zinc test. After standing 24 hours in this reagent there is a slight cellulose reaction in the walls of the vessels, tracheids, and wood fibers but none in the medullary rays. In making free-hand sections of the diseased wood the medullary rays and vessels are easily ruptured, owing to the thinning and weakening of the walls by the solvent action of the fungus.

The concentric splitting of the rotted wood usually occurs in the zone of the larger vessels, which are weakened by the corrosion of their bordered pits and walls. The longitudinal splitting is caused by the coalescence of the enlarged bordered pits of the tracheids and the thinned walls of the medullary rays. The discolored areas seen in the earlier stages of the rot are due to the presence in the cells of the medullary rays, wood parenchyma fibers, and sometimes in the lumen of the wood fibers of a brownish liquid, which disappears before the white stage of the rot is reached. In the final stage of the rot the wood is somewhat spongy in texture and when dry is easily crushed between the fingers.

Old sporophores were often found at different places on the collar of the diseased tree, due probably to the gradual rotting of the roots upward toward the stool of the tree and the formation of sporophores whenever a rotted area reached the collar of the tree or the underside of a root whose upper surface was exposed to the air. The sporophores are usually attached to the trunk of the tree at the surface of the ground, but they were also found on the exposed roots or even in rare cases on the ground, having been produced from hyphæ issuing through the soil from diseased roots lying a short distance below. Only one sporophore was found on the trunk at a distance above the collar of the tree, and in this case two trees had grown together at the butts for a distance of 12 inches. The rot had extended from the diseased roots upward in the injured sapwood of the oak along the juncture of the two trunks, and a small sporophore had formed 10 inches from the ground.

The sporophores when old and mature usually have a hard fibrous-corky to corky-woody context and a very rough, uneven, tuberculate upper surface, owing to the leaves, twigs, and other foreign substances falling on the upper surface of the growing pileus. (Pl. XXII, fig. 4.) After weathering for some months, the color of the pileus is a chestnut brown or sometimes becomes almost black and rimose. The old sporophores as a rule are partially destroyed by insects, especially the subhymenial layer and the adjacent ends of the pores. Portions of the outer pore surface, the central part of the context, and the base of the sporophores usually persist and can be found attached to the bases of the diseased trees for several years after maturity.

The mouths of the pores in the weathered sporophores are stuffed to a depth of 0.5 to 1 millimeter with a firm, brown mycelial mass, thus completely hiding all trace of the pores from a surface view. This stuffed pore layer becomes hard and brittle and gradually cracks in weathering and peels off from the deeper and more insect-eaten portion. Immature specimens shipped before being thoroughly desiccated have the tubes loosely stuffed with a delicate, white arachnoid mycelium, which appears on the spore surface as a thin creamy layer about 0.5 of a millimeter thick. This condition is probably due to a growth developed in the sporophore while in transit in a damp state. The stuffed mouths of the pores in old weathered sporophores is apparently a normal state of old specimens from certain sections of the United States. However, this stuffed condition of the pores in old sporophores is not always present, as several specimens both from America and Europe were seen by the writer in which the mouths were entirely free and open.

The tubes in all the specimens examined—both American and European—contain characteristic setæ. They are dark chestnut brown, thick walled, curved, cat's claw to hawk beaked in shape, giving them a somewhat bulbous-shaped base when seen in side view. They are 7 to 12 $\mu$  thick at base, 15 to 24 $\mu$  long, and usually project 10 to 20 $\mu$  beyond the hymenial surface into the tube cavity.

The sporophores vary greatly in shape and size, ranging from 9 cm. long, 5 cm. wide, and 1½ cm. thick to 20 cm. long, 15 cm. wide, and 10 cm. thick, and may be simple or imbricated, depending to a great extent on the environment and food supply. In many of the thick sporophores growing from the collar of the tree the pore surface is borne at an angle of 40° to 60° to a horizontal plane. In the thinner and broader specimens the pore surface approaches more nearly the normal angle of other dimidiate sporophores. The margin is very thick and rounded in most of the specimens. The cavities left in the upper surface of the pileus by the drops of water which exude during the rapidly growing period of the sporophore are plainly discernible even in many of the old sporophores. The pore surface extends entirely to the point of attachment to the substratum even when the sporophore has a rounded substipe, as is often the case when it forms on the upper surface of exposed roots.

When sporophores are developed at the collar of trees growing in sandy land, the soil for 4 to 6 inches wide and 2 to 3 inches deep immediately at the base of the sporophore is often cemented into a hard, compact, bricklike mass, apparently by hyphæ, as many colorless fungous threads were found ramifying through it.

*Polyporus dryadeus* has been found attacking the roots of *Quercus texana* Buckl. and *Q. nigra* L. in eastern Texas. Some of the diseased trees were dying, while others were evidently in poor health. It has been found on *Q. alba* L. and *Q. velutina* Lam. in the Ozark National Forest, of Arkansas. The majority of the trees in the Ozarks affected with the disease caused by *P. dryadeus* were growing on sandy ridges and southern slopes where the soil was thin and conditions were unfavorable to rapid, vigorous growth. Two trees of *Q. minor* (Marsh) Sarg. were found with this disease in Oklahoma; one was dead and the other in apparently fair health.

*Polyporus dryadeus* also occurs in *Q. alba* L., *Q. rubra* L., and *Q. prinus* L. in Virginia, where seven trees were found with this rot; five were growing in crowded, unfavorable conditions, while one was standing at some distance from other trees and was apparently in good health. Yet at least two large roots of this lone tree—a white oak—were thoroughly rotted, while sporophores were found on three sides of the tree, one growing from the top of an exposed root. This sporophore was over 1 foot tall and at least as wide, judging from the old weathered remains. It was from this root that figure 5 of Plate XXII was taken. Of the five crowded trees one was much suppressed and would probably have died in a year or two. This tree was dug up, and studies were made of its roots, stool, and trunk. All of its roots, except three large lateral ones which ran near the surface of the ground, were completely rotted by *P. dryadeus*. The three living roots were partially rotted on the lower side and at the ends, but were still alive and strong enough to hold the tree in the ground. Old sporophores were found on all sides of this tree at the ground line.

The trees of *Quercus prinus* which were attacked by this root rot were found by Mr. G. F. Gravatt, of the Office of Investigations in Forest Pathology, who made the following statement concerning the diseased trees:

Early in July at Bluemont, Va., three small trees of *Quercus prinus* were found which had been killed while in full leaf and which from a distance were mistaken for chestnut trees that had been girdled by the chestnut bark disease (*Endothia parasitica*). Whitish spots of mycelium were found on the bark of nearly every root, while the lower portions of the roots were so thoroughly rotted that the two smaller trees were easily pulled up by hand. The two small trees were somewhat suppressed, but the largest (3½ inches in diameter) was situated in an open space in the woods. These three trees were about 100 yards distant from each other.

The writer examined the rot from the roots of these diseased trees and found that it was caused by *Polyporus dryadeus*.



Four trees of *Quercus alba* were found affected with this disease in Maryland. All had been uprooted by the wind, two very recently, so that the character of the earlier stages of the rot and its progress in the roots was easily observed. In both of these trees the rot was only in the lower ends of the roots and had not reached the stool nor formed sporophores. Three of these uprooted trees were growing in dense stands and were much suppressed.

Oaks which have been uprooted by the wind may be separated into two classes: (1) Those whose root system has been weakened by insect or fungous attack and (2) those with a very shallow root system, due to the presence of impermeable layers of rock in the subsoil or to the groundwater being constantly near the surface of the ground (within 1 to 2 feet). Trees uprooted by wind owing to rotten roots have very little soil adhering to the upturned stool of the tree, as most of the roots break off within 1 to 2 feet of the base of the tree. On the other hand a tree with a sound root system brings with it when uprooted a large mass of earth several cubic yards in size. Bearing this in mind one can often distinguish, even at a distance, wind-thrown trees with sound roots from those overthrown on account of root-rot.

In every instance where the sporophores of *Polyporus dryadeus* were found on trees the roots were diseased with the same type of root-rot. In wind-thrown trees where the disease was not far enough advanced to produce sporophores the rot was identical with that obtained from the roots of trees which had sporophores of *P. dryadeus*. The rot in such uprooted trees evidently began at some point on the lower end of the roots and advanced up the roots toward the base of the tree, stopping, however, when it reached the surface of the ground. Roots lying very near the surface of the soil, especially large ones with their upper surfaces exposed to the air, are not entirely rotted or even killed by this fungus. Many instances of such superficial roots were found in which the part underground was rotted while the upper portion remained alive. The cross section of the root illustrated in Plate XXII, figure 6, shows the upper part alive, while the lower and more deeply buried portion is rotted. This root forked some 2 feet from the tree; one root, 10 inches in diameter, went down deep in the soil and was thoroughly rotten and dead; the other fork was 2 to 4 inches deep and was perfectly sound 2 feet from where the rotted root joined it.

The inability of the fungus to rot exposed roots and the trunk above the surface of the soil, coupled with the further fact that the sporophores usually are attached to what superficially appears to be sound wood, probably explains why the connection between this rot and the fungus causing it has not been previously noted. Trees in all stages of this disease were seen; some were already dead, others dying, others on the decline, while some showed no evidence of the disease until they were overthrown by the wind and the decayed roots were exposed. Some of

the trees bearing sporophores were apparently in a healthy condition, yet an examination of the root system showed in every case one or more large roots completely rotted. Two stumps of *Quercus alba* were found with sporophores of *Polyporus dryadeus* springing from the rotted roots. In no instance were trees which were attacked by this fungus found in groups or even adjacent to each other. The majority of the trees with this disease in their roots were growing under unfavorable environments. The boles of some of them were also attacked by various heart-rotting fungi, while others were perfectly sound above the collar, although they bore sporophores of *P. dryadeus* at the ground line.

No rhizomorphs of any kind were found associated with this rot, either beneath the bark, on the surface of the roots, or ramifying in the adjacent soil. How the lower part of the smaller roots became infected is not known.

The identity of the fungus causing this root-rot with the European fungus known as *Polyporus dryadeus* may be questioned. Through the courtesy of the officials in charge, the writer was permitted to examine all the American and European specimens of *P. dryadeus* in the following herbaria:

Pathological and Mycological Collections of the Department of Agriculture, at Washington, D. C., Herbarium of the New York Botanical Garden, and the Cryptogamic Herbarium of Harvard University.

Authentic specimens of *Polyporus dryadeus* from America, England, France, Germany, and Austria were examined, and a careful comparison of each with the material used as the basis of this article showed that the American plant under discussion is undoubtedly identical with the European fungus known as *P. dryadeus*.

There are three collections in the laboratory of the Office of Investigations in Forest Pathology, at Washington, D. C., of a *Polyporus* on *Tsuga heterophylla* from three widely separated localities in the State of Washington. These specimens were collected by C. J. Humphrey, of this office, and the legends accompanying them indicate that the sporophores were attached to the host at or near the surface of the ground and that the plant is a true parasite that kills the trees it attacks. These specimens agree in all essential characters, both gross and microscopic, with *Polyporus dryadeus*, and although the writer has not seen the rot produced in this host, he believes the fungus is this plant.

#### SUMMARY

(1) *Polyporus dryadeus* is a root parasite of the oak, producing a white sap rot and a heart rot in the roots.

(2) In all the trees examined this rot did not extend upward into the tree as a true heart or sap rot of the trunk, but was limited to the underground parts of the tree.

(3) The rot and sporophore described and figured by Robert Hartig do not belong to *Polyporus dryadeus*, but to *Polyporus dryophilus*.

(4) In the majority of cases only old or much suppressed trees or trees growing under very unfavorable conditions were found attacked by this disease.

(5) The disease does not seem to spread readily to adjacent trees.

(6) The disease is widely distributed both in America and in Europe and is probably found in these countries throughout the range of the oak.

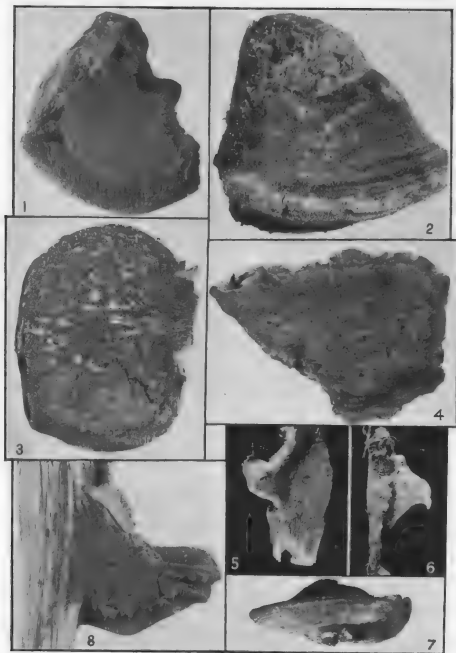
#### LITERATURE CITED

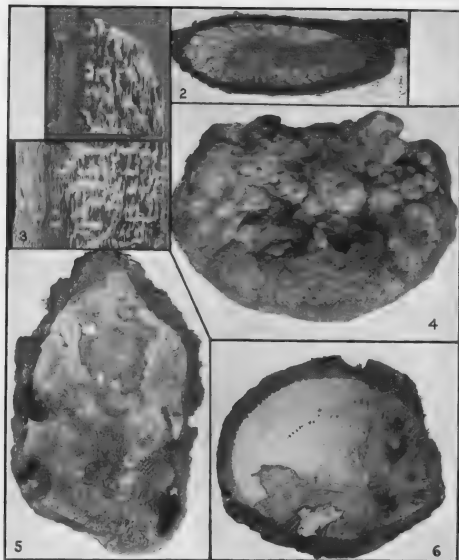
1789. BULLIARD, PIERRE. *Herbier de la France*. Paris. pl. 458.  
1791. ———. *Histoire des Champignons de la France*. t. 1, Paris. p. 356, pl. 458.  
1799. PERSEON, C. H. *Observationes Mycologicae*. pars 2, Lipsiae. p. 3.  
1801. ———. *Synopsis Methodica Fungorum*. pars 2, Göttingae. p. 537.  
1821. FRIES, ELIAS. *Systema Mycologicum*. v. 1, Gryphiswaldiae. p. 374.  
1849. HUSSEY, MRS. T. J. *Illustrations of British Mycology*. London. [ser. 1], pl. 21.  
1878. HARTIG, ROBERT. *Die Zersetzungserscheinungen des Holzes der Nadelholzbäume und der Eiche*. Berlin. p. 124-128, pl. 17.  
1897. TUBEUF, KARL VON. *Diseases of Plants Induced by Cryptogamic Parasites*. English edition by W. G. Smith. London. p. 440-442, fig. 272-274.  
1910. MASSEE, GEORGE. *Diseases of Cultivated Plants and Trees*. London. p. 380-381.  
1910. HEDGCOCK, GEORGE G. Notes on some diseases of trees in our national forests. [Not published.] *Abstract in Science*, n. s., v. 31, p. 751.  
1912. ———. Notes on some diseases of trees in our national forests.—II. Phytopathology, v. 2, no. 2, p. 73-74.  
1913. ———. Notes on some diseases of trees in our national forests.—III. Phytopathology, v. 3, no. 2, p. 111-114.  
1913. LLOYD, C. G. Letter No. 44. p. 8, note 47.



## DESCRIPTION OF PLATES

- PLATE XXI. Fig. 1.—*Polyporus dryophilus*: A median-longitudinal section of a sporophore on *Quercus alba* from Arkansas, showing the granular core and the white mycelial lines in the central and rear portion.
- Fig. 2.—*Polyporus dryophilus*: Side view of the ungulate type of sporophore on *Quercus californica* from California.
- Fig. 3.—*Polyporus dryophilus*: Median-longitudinal section of the globose type of sporophore on *Quercus garryana* from California, showing the large granular core and prominent white mycelial lines.
- Fig. 4.—*Polyporus dryadeus*: Median-longitudinal view of a young sporophore on *Quercus texana* from Texas, showing the fibrous, non-granular nature of the context.
- Fig. 5.—*Polyporus fulvus* Fries: Median-longitudinal view of a sporophore on *Quercus* sp. from Sweden, showing the granular core characteristic of *P. dryophilus*.
- Fig. 6.—*Polyporus vulpinus*: Median-longitudinal view of sporophore on *Populus* sp. from Sweden, showing the granular core characteristic of *P. dryophilus*.
- Fig. 7.—*Polyporus dryophilus*: Front view of the applanate type of a sporophore on *Populus tremuloides* from Colorado, showing the faint zones on the pileus where the hairs have disappeared.
- Fig. 8.—*Polyporus dryophilus*: Median-longitudinal view of sporophore on *Populus tremuloides* from Colorado, showing the granular core originating between the sapwood and bark and extending into the center of the sporophore.
- XXII. Fig. 1.—*Polyporus dryophilus*: Radial-longitudinal view of the rot occurring in *Quercus* sp. from Europe and said to be the rot produced by *P. dryadeus*.
- Fig. 2.—*Polyporus dryadeus*: Cross section of a small root of *Quercus alba* from Maryland, showing the mottled appearance of the diseased wood in the middle stages of the rot.
- Fig. 3.—*Polyporus dryophilus*: Radial-longitudinal view of the rot appearing in *Quercus alba* from Arkansas, showing the advancing line of rot in a branch.
- Fig. 4.—*Polyporus dryadeus*: Upper surface of a sporophore on roots of *Quercus texana* from Texas, showing the rough tuberculate pileus.
- Fig. 5.—*Polyporus dryadeus*: Rot occurring in an apparently sound root of *Quercus alba* from Virginia, showing cross section of a diseased root, immediately adjacent to the point of attachment of a large sporophore of *P. dryadeus*, 1 foot high and 1 foot wide. Some sound, living wood is still present.
- Fig. 6.—*Polyporus dryadeus*: Cross section of diseased root of *Quercus alba* from Virginia, showing the nearly sound, living upper half of the root and the badly diseased lower half.





# THE FOOT-ROT OF THE SWEET POTATO

By L. L. HARTER,

*Pathologist, Cotton and Truck Disease and Sugar-Plant Investigations,  
Bureau of Plant Industry*

## INTRODUCTION

On August 9, 1912, Mr. O. H. Weiss sent the writer some diseased sweet-potato (*Ipomoea batatas*) vines from the vicinity of the Dismal Swamp, Va., with a request for information regarding the nature of the trouble. The stems for a short distance above the ground were covered with black fruiting bodies of a fungus, and suggested macroscopically the conidial stage of *Diaporthe batatas*, the cause of the sweet-potato dry-rot. Careful examination of the material showed that in structure these fruiting bodies differed from those of the dry-rot organism, although it was apparent that both fungi belonged to the same general group. The organism was isolated in pure cultures from material taken from diseased sweet-potato stems and its parasitic habits and growth in artificial cultures compared with the dry-rot organism.

On August 22, 1912, the writer visited the sweet-potato fields near the Dismal Swamp in order to observe the disease under natural conditions and to ascertain the extent of the loss. The disease was found in practically every field, causing a loss of from 10 to 50 per cent of the crop, and in exceptional cases even more.

During August, 1913, the disease was found for the first time in many fields near Cape Charles and Keller, Va. Whether this is the first appearance of the disease in this part of the State is not known. The writer had inspected many fields in this section for several summers previous to 1913 and never observed the disease. It seems likely, therefore, that the disease is either new to these places or has heretofore occurred only to a very limited extent. The organism was isolated from specimens collected at both Cape Charles and Keller, and it was found to be identical with the one obtained during 1912 and 1913 from the vicinity of the Dismal Swamp.

Inquiry among the farmers in the vicinity failed to give a definite idea as to how long the disease has been prevalent. It was learned, however, that the disease has increased in severity in the last few years, and if not checked is likely to prove a serious handicap to the growing of a crop that would otherwise be a profitable industry.



## DIAGNOSIS OF FOOT-ROT

The foot-rot organism is a slow-growing parasite, especially during the earlier stages of infection. During about the first three weeks after inoculation, only a slight enlargement of the wound in all directions takes place. About three weeks seems to be required for the fungus to overcome the plant sufficiently to cause any marked reduction in its vitality or vigor. As soon, however, as the fungus gets the upper hand, it develops very rapidly and in about one week more completely girdles and extends along the stem from 2 to 5 inches, killing the plant by the destruction of the cortex. At the end of about another week wilting of the leaves is first observed, the plants beginning to die soon afterwards. There is considerable variation in the length of time a plant will live after becoming infected, especially under greenhouse conditions, some of the plants dying in three or four weeks, while others may survive for one to four weeks longer. It is also interesting to note in this connection that those plants appearing to be the strongest when inoculated are likely to be the first to succumb to the disease. An explanation of this may be that a vigorously growing plant may stimulate the fungus to more rapid development.

The first sign of the disease of inoculated plants is a blackening of the cortex of the stem at the point of inoculation. When inoculated at the soil line, the fungus seldom grows more than half an inch below the surface of the ground, but it extends up the stem several inches. The leaves near the point of inoculation are invaded and soon turn yellow and fall off. Under greenhouse conditions numerous black pycnidia break through the epidermis of the stem (Pl. XXIII, fig. A) along the blackened area about the time the foliage begins to wilt. Under natural conditions in the field, on the other hand, the pycnidia form on the invaded tissue before the wilting of the plant. It was observed also that diseased plants will survive under field conditions much longer than in pots in the greenhouse, where they are naturally handicapped by artificial conditions. Many diseased plants in the field with fruiting bodies abundantly formed on the stem are often sustained by the roots which are thrown out at the nodes along the stems, although the main stem may be nearly destroyed by the fungus. If not supported by roots at the nodes, the diseased plants readily succumb.

As a rule, the disease is confined to the stem of the plant from the soil line to 4 or 5 inches above it. However, at Cape Charles, Va., in some of the low, rather wet fields, where there was a rank vegetative growth, vines were found diseased several feet from the hill. In such cases infection evidently took place at the node and spread in each direction (Pl. XXIV), the vine on each side of the diseased area remaining healthy. The organism isolated from pycnidia on such diseased spots was identical with the one obtained from the stem.

## CAUSE OF THE FOOT-ROT

The organism causing the foot-rot of the sweet potato has been described as *Plenodomus destruens*.<sup>1</sup> It has also been pointed out that the fungus does not fit well into this genus or into any of the present-known genera. At the time, however, it was thought better to describe it as a new species of the genus *Plenodomus* rather than to create a new genus in a group where there are already a great many genera. It is probable that this organism is the conidial stage of an ascomycete which will eventually be discovered, and in view of that fact its generic position can only be temporary. It falls naturally in the order Sphaeropsidales and is more closely related to *Phoma*, *Phomopsis*, and *Phyllostycta* than to any of the other genera in the order.

The diagnosis of the genus *Plenodomus* as found in Saccardo's *Sylloge Fungorum* is somewhat brief. In 1911 Diedicke<sup>2</sup> worked over this genus, describing it more fully and pointing out the characteristics which distinguish it from *Phomopsis*, the genus with which it is most likely to be confused.

Since it is quite evident that the foot-rot fungus is not a *Phoma*, differing from that genus (1) in having more irregularly shaped pycnidia (Pl. XXV, B) and (2) in having a well-defined beak (Pl. XXV, A), attention will be given only to the characteristics which distinguish the foot-rot organism from *Phomopsis*, the conidial stage of the sweet-potato dry-rot.

According to Diedicke, *Plenodomus* is characterized by having only two walls composing the pycnidium—a dark outer wall and a hyaline one within. The outer wall completely surrounds the pycnidium and is of uniform thickness at the top and base. The inner hyaline layer is composed of several layers of cells and is somewhat thicker than the outer wall. The conidiophores are short, fragile, and inconspicuous. The spores are rounded at both ends.<sup>3</sup>

On the other hand, the pycnidium of *Phomopsis*, according to the same author, is composed of four walls. The upper portion of the pycnidium, especially about the beak, is composed of thick black cells. The dark color of this layer of cells becomes less conspicuous in the lower portion and practically disappears at the base of the pycnidium. *Phomopsis* is further characterized by the development of a stroma and chambering of the pycnidium. The conidiophores are long, conspicuous, and awl-shaped, and the spores are spindle-shaped. Because of the variation in the shape of the spores this latter character is of less importance than some of the others in separating the genus from *Plenodomus*. Stylospores are found in some species of *Phomopsis*.

<sup>1</sup> Harter, L. L. Foot rot, a new disease of the sweet potato. *Phytopathology*, v. 3, no. 4, p. 243-245, 2 fig., 1913.

<sup>2</sup> Diedicke, H. Die Gattung *Plenodomus* Preuss. *Ann. Mycol.*, Jahrg. 9, No. 2, p. 137-141, pl. 8, 1911.

<sup>3</sup> This last character is perhaps of the least importance, since it is well known that the spores vary greatly within the genus and even in the same species. In fact, the spores of some species of *Phomopsis* have rounded ends.

It will be seen, therefore, that the following characteristics belonging to the dry-rot fungus are not found in the foot-rot organism: (1) Stroma; (2) chambering of the pycnidium; (3) conidiophores conspicuously long and awl-shaped; and (4) long, filiform, hook-shaped stylospores.

What is believed to be even more significant than the differences in morphological characters between these two organisms is the difference in parasitic habits and growth in artificial cultures. It has been pointed out in a previous bulletin<sup>1</sup> that the dry-rot fungus does not kill the plant but lives in apparent harmony with it without injury. The pycnidia appear on the stem only after the plant has been lifted and kept in a damp chamber for 10 days or 2 weeks, this being the first evidence that the plant was infected. The organism occurs on the petioles and leaves of dead plants and often develops on apparently sound roots after a period of time in storage. Stylospores are frequently found on the roots and stems.

The foot-rot disease, on the other hand, kills the plant in three to eight weeks after infection by the destruction of the cortex of the stem for several inches above and a little distance below the surface of the soil. Pycnidia are formed on the diseased portion of the stem about the time the foliage begins to wilt (Pl. XXVI, fig. A), and under field conditions even earlier.

The growth of the organism on several kinds of the commonly used artificial media and especially on synthetic agar<sup>2</sup> and on corn meal<sup>3</sup> furnishes additional means of distinguishing the two diseases.

On synthetic agar the foot-rot fungus grows slowly and under normal conditions forms a very compact growth, at first irregular in outline with a slightly darker center, attaining a diameter of not more than 2 or 3 mm. at the end of a week or 10 days. (Pl. XXVII, fig. B.) On the same culture medium the dry-rot fungus grows much faster, forming a loose, flaky growth of uniformly white hyphæ having an irregular outline. (See Pl. XXVII, fig. A.) The growth of the dry-rot fungus is so loose and inconspicuous that it is scarcely visible until it has attained a diameter of 2 or 3 mm.

<sup>1</sup> Harter, L. L., and Field, Ethel C. A dry rot of sweet potatoes caused by *Diaporthe batatatis*. U. S. Dept. of Agr., Bur. Plant Indus., Bul. 281, 38 p., 4 pl., 1913.

<sup>2</sup> Synthetic agar is prepared as follows:

	Grams.
Distilled water.....	1,000
Dextrose.....	200
Peptone (Witte's).....	10
Ammonium nitrate.....	10
Potassium nitrate.....	5
Magnesium sulphate.....	2.5
Calcium chlorid.....	0.1
Agar agar.....	20

Place the water in the beaker first; then add other ingredients in the order given. Stir and let stand till the agar agar is moist. Steam 1 hour. Tube with constant stirring. Plug and autoclave for 15 minutes at 110° C. Agar of high purity only should be used.

<sup>3</sup> Corn-meal flasks are prepared as follows: Place 5 grams of corn meal in a 100 c. c. flask. Add 45 c. c. of distilled water and steam for 15 minutes. Plug and autoclave at 11 pounds pressure for 20 minutes.

On corn meal the dry-rot organism forms a black stroma composed of several pycnidia with long exerted beaks. The stroma is  $\frac{1}{2}$  to 1 or more mm. in diameter and is preceded by a profuse growth of mycelia. The foot-rot organism, on the other hand, forms no stroma on corn meal. The pycnidia stand separately and are very numerous, while the mycelial growth is slight and inconspicuous. The pycnidia follow closely after the growth of hyphæ, the pycnidial zone increasing with the increase in diameter of the mycelial growth. Spores are exuded in great quantities, forming a yellowish transparent liquid over the surface of the medium.

#### ISOLATION OF THE FUNGUS

Pure cultures of the foot-rot organism were particularly easy to secure by the poured-plate method. Stems on which the pycnidia were present were thoroughly washed in hydrant water or, preferably, disinfected with mercuric chlorid for about 40 seconds and then rinsed in sterile water. A few of the pycnidia were then macerated in a watch glass in sterile water and one or two loopfuls transferred to tubes of synthetic agar and plates poured. The fungus grows very slowly on agars, particularly on synthetic agar. The colonies are not visible in the plates for three days and often not until five or six days after they are made. Because of the characteristic growth on synthetic agar the organism can easily be picked out from other fungi when the appearance of the colony is once known.

#### DESCRIPTION OF THE FUNGUS

MYCELIUM.—The appearance of the mycelium varies so markedly on different culture media and according to the age of the culture that it would be difficult to give a simple, characteristic, general description. In young cultures and for the most part in old cultures it is nearly always hyaline, although occasionally browned hyphæ may be found. Oil globules are found in the mycelia at all ages (Pl. XXV, C). Hyaline, spherical and oval, thick-walled bodies 8 to  $13\mu$  in diameter, generally filled with oil globules, intercalated or, rarely, terminally, in chains or singly (Pl. XXV, D), occur in most media and at nearly all ages. Browned bodies morphologically similar to the hyaline ones but occurring mostly at the end of the hyphæ (Pl. XXV, E) are frequently found in older cultures. In 7-months-old corn-meal cultures which were quite well dried out the brown bodies were abundant, especially where the media came in contact with the glass. In these cultures the hyaline forms were few. In 4-months-old cultures of string beans brown and hyaline bodies and brown hyphæ were present. The brown hyphæ were filled with numerous beadlike swellings. On the other hand, in a rice culture of the same age only hyaline hyphæ and hyaline spherical or oval bodies were found.

PYCNIDIA.—The pycnidia are at first buried, but later break through the epidermis, appearing as black dots scattered over the surface. They stand close together on the stem and roots, but they are not confluent

or only rarely so. (Pl. XXIII, fig. A.) They are irregular in form and vary greatly in size, averaging about  $300\mu$  through their greatest diameter.

In cross section the pycnidia from the stem and roots show somewhat different structures. From either source they are completely inclosed by a dark, almost black, outer wall (Pl. XXV, A and B).

The pycnidia on the roots have a well-defined inner hyaline layer almost equal in thickness to the outer wall (Pl. XXV, A). On the stem the dark wall is more conspicuous, being better developed than on the root, and the inner hyaline layer is completely lacking (Pl. XXV, B).

The basidia are short, fragile, somewhat inconspicuous, and arise from the inner hyaline layer or from the dark wall in pycnidia where the hyaline layer is absent. They are 6 to  $13\mu$  in length and very narrow.

The spores are discharged through a beak varying somewhat in length, which may arise from any part of the upper surface of the pycnidium. In old dried specimens the upper portion of the pycnidium may fall away.

PYCNOSPORES.—The pycnospores are oblong, rounded at both ends, 6.8 to  $10.0\mu$  long by 3.4 to  $4.1\mu$  wide, with two large oil droplets. They are hyaline, 1-celled, and sometimes slightly curved (Pl. XXV, F).

In the same pycnidium on the host and occasionally on rice and on sweet-potato-stem cultures are found in addition to the pycnospores hyaline curved or straight bodies 6 to  $15\mu$  in length. These bodies are somewhat cylindrical in shape and rounded or tapering at the ends (Pl. XXV, G). The function of these bodies is not known. Several attempts have been made to germinate them, and while there have been some reasons to believe that a germ tube was developed, this point was not definitely settled. These bodies were formed so sparingly in artificial media that it was necessary to use those from the host in order to test their germination in hanging-drop cultures in Van Tieghem cells. Because of the difficulty in sterilizing this material, bacteria completely overran the cultures in about 24 hours, thus terminating the experiment.

## PARASITISM OF THE ORGANISM

### INOCULATION EXPERIMENTS

The details of inoculations with *Plenodomus* are found in the following pages. For convenience, the experiments are numbered and arranged according to dates of inoculation and under the heading to which they belong. The organisms used to make the inoculations are also designated by numbers.<sup>1</sup>

<sup>1</sup> For convenience and ready reference, separate numbers (100, 101, 102, 108, and 110) were given to the different isolations where they or subcultures from them were used for inoculations. No. 100 was given the organism obtained from specimens sent the writer Aug. 9, 1912, and No. 101 from specimens collected Aug. 22 from the same locality. The other numbers used, 102, 108, and 110, were given to the organism reisolated from inoculated plants. A new number was given the fungus only when it was the source from which other plants were to be inoculated. However, it should be kept in mind that these different numbers represent only different isolations of the same organism (*Plenodomus destruens*).

Most of the inoculations were made in the greenhouse, principally because they were performed in the winter. One set, however, which was conducted in the field, gave results so similar to those in the greenhouse that it was not possible to distinguish between them in any essential details. The plants for inoculation were obtained from sound potatoes carefully selected for the purpose. They were grown in pots of sterilized soil and kept far enough apart to prevent accidental infection from watering and overlapping of the vines. Only strong, vigorously growing plants were inoculated, all others being thrown out. That there was probably no accidental infection is shown by the fact that not a single check in the whole series of inoculations became diseased.

#### INOCULATIONS IN THE FIELD

EXPERIMENT NO. 1.—On August 26, 10 sweet-potato plants, the vines being about 3 feet long, were inoculated <sup>1</sup> on the Potomac Flats near Washington, D. C., by inserting pycnospores and hyphæ of organism No. 102 (culture No. 1 of Aug. 15) into the lower part of the stem. Ten plants pricked with a sterile needle were used as checks.

Results.—On September 18 all the inoculated plants were infected,<sup>2</sup> the plants turning yellow, and the lower leaves dropping off. The periphery of the stem for 3 to 5 inches above the ground was black, and pycnidia were abundantly formed thereon. The stems were blackened throughout, but attempts to isolate the fungus from the fibro-vascular bundles gave negative results. None of the checks were diseased. The infected plants were all lifted on October 10, taken to the laboratory, and examined. Pycnidia were present on all. On October 12 cultures were made from seven of these plants, and the organism recovered <sup>3</sup> in each case.

#### INOCULATIONS IN THE GREENHOUSE

EXPERIMENT NO. 2.—On August 26, 1912, 10 young sweet-potato plants in pots were inoculated with organism No. 100 (culture No. 8 of Aug. 15) by inserting pycnospores and hyphæ into the stem at the soil line. Five plants pricked with a sterile needle were left as checks.

Results.—On September 16 four plants, on November 14 one, and on November 25 three, or a total of eight plants, were infected. None of the checks were diseased. Pycnidia were formed on all the diseased plants and the organism recovered from three. The experiment was terminated December 2, 1912.

EXPERIMENT NO. 3.—On November 13 ten young sweet-potato plants in pots were inoculated as in experiment No. 2 with organism No. 101 (culture No. 9 of Oct. 31). Six plants pricked with a sterile needle were left as checks.

Results.—On December 14 one plant, on December 18 three, on December 21 one, on December 26 one, on December 30 two, and on January 10 two, or a total of ten plants, were infected. Pycnidia were present on eight plants when lifted and developed on the other two after two days in a moist chamber. All the checks remained healthy. The experiment was terminated January 17, 1913.

<sup>1</sup> All inoculations recorded in this article, unless otherwise stated, have been made from cultures grown on sterile moistened corn meal and only when spores were exuding from the pycnidia.

<sup>2</sup> By "infected" is to be understood the stage when the plant began to wilt and die. It was generally quite evident some days earlier that the plants were infected, although they were not so recorded until this stage was reached.

<sup>3</sup> No attempt has been made to recover the organism from all diseased plants. Occasionally, however, the fungus was recovered from infected plants in order to compare it with the original strain, or for the purpose of inoculating it into other plants.

EXPERIMENTS NOS. 4, 5, 6, AND 7.—On November 18 four sets of inoculations were made of 10 plants each (40 plants in all) with organism No. 101 (culture No. 8 of Oct. 31), as follows: (No. 4) By smearing pycnospores on the leaves and spraying the foliage with spores suspended in sterile water and covering the plants with bell jars for 24 hours, (No. 5) by smearing pycnospores on the base of the stem, (No. 6) by pouring pycnospores suspended in sterile water about the plants, and (No. 7) by inserting pycnospores and hyphæ into the base of the stem. Six plants were left as checks.

Results.—(No. 4) No infection. (No. 5) On December 30 one plant, on January 8 one, on January 13 two, on January 15 one, on January 23 one, and on January 30 one, or a total of seven plants, were infected. Pycnidia were abundant on all when lifted. (No. 6) On December 26 one plant, on December 30 one, on January 4 two, on January 6 one, on January 10 one, and on January 13 one, or a total of seven plants, were infected. The infected plants were lifted on January 24 and pycnidia were present on all. (No. 7) On December 21 two plants, on December 26 one, on December 28 two, on January 6 one, on January 11 one, on January 13 one, on January 14 one, and on January 17 one, a total of ten plants, or all of those inoculated, were infected. Pycnidia were present on nine of these plants when lifted and developed on the other one after three days in a moist chamber. None of the checks were diseased. The experiment was terminated on February 27.

EXPERIMENT NO. 8.—On December 9 six 5-weeks-old sweet-potato plants in pots were sprayed with pycnospores and hyphæ of organism No. 101 (culture No. 1 of Nov. 12) suspended in sterile water. The plants were covered with bell jars and shaded with paper for 24 hours. Six plants were left as checks.

Results.—No infection. The experiment was terminated February 27, 1913.

EXPERIMENT NO. 9.—On December 28 eight 4-months-old sweet-potato plants grown in pots were inoculated by inserting pycnospores and hyphæ of organism No. 100 (culture No. 2 of Dec. 10) into the base of the stem. Six plants pricked with a sterile needle were left as checks.

Results.—On January 23 one plant, on February 4 one, on February 7 three, and on March 8 one, or a total of six plants, were infected. The checks remained healthy. Pycnidia were present on all the infected plants when lifted. The organism was recovered from two of the infected plants. The experiment was terminated March 27.

Only young plants were used in the first eight experiments. Experiment No. 9 was made with old plants (as compared with those used in experiment No. 8) for the purpose of determining whether they were as susceptible as young ones to the foot rot. The results indicate that they are.

EXPERIMENT NO. 10.—On January 23, 1913, six sweet-potato plants (three old and three young) grown in pots were sprayed with pycnospores of organism No. 100 (culture No. 3 of Dec. 28) suspended in sterile water. All the plants were making a good growth. As soon as the plants were sprayed, they were covered with bell jars and manila paper for 48 hours. Six plants were left as checks.

Results.—None of the plants were infected. The experiment was terminated March 27, 1913.

EXPERIMENTS NOS. 11 AND 12.—On January 17 ten young plants, each of *Ipomoea purpurea* (L.) Roth. and *Ipomoea hederacea* Jacq. were inoculated with organism No. 100 (culture No. 4 of Dec. 28). Seven plants were left as checks.

Results.—No infection.

EXPERIMENT NO. 13.—On December 2 five young plants of *Ipomoea coccinea* L. in pots were inoculated at the base of the stem with organism No. 101 (culture No. 2 of Nov. 12). Five plants were left as checks.

Results.—On February 28, 1913, three plants were infected. The organism from two of the plants was recovered by pouring plates from the pycnidia and from the third plant by planting bits of diseased tissue in plates of synthetic agar.

None of the checks became diseased. The experiment was terminated February 28, 1913.

EXPERIMENT NO. 14.—On May 9 seven sweet-potato plants in pots in the greenhouse were inoculated by inserting the hyphæ (no pycnidia in the culture) of organism No. 101 (culture No. 2 of May 5) into the lower part of the stem. Six plants were left as checks.

Results.—On May 31 six plants, and on June 4 one, or a total of seven plants, were infected. None of the checks were diseased. When the experiment was terminated on June 5, pycnidia were abundant on the stems of all diseased plants.

EXPERIMENT NO. 15.—On September 3 six sweet-potato plants in pots in the greenhouse were inoculated by inserting spores and hyphæ of organism No. 101 (culture No. 13 of Aug. 14) into the vine at the node 3 to 4 feet from the hill. Five other vines were wounded with a sterile needle and left as checks.

Results.—On September 25 five of the vines were infected at the point of inoculation. The organism had spread 2 inches or more each way from the point of inoculation. None of the checks were diseased. The experiment was terminated October 5, 1913.

EXPERIMENT NO. 16.—On September 3 five sweet-potato plants in pots in the greenhouse were inoculated by inserting spores and hyphæ of organism No. 108 (culture No. 17 of Aug. 18) into a vine at the node 3 to 4 feet from the hill. The checks were the same as those used in experiment No. 15.

Results.—On September 25 all the vines were infected at the point of inoculation, the organism spreading as in experiment No. 15. The experiment was terminated October 5, 1913.

#### INOCULATIONS FROM REISOLATIONS

EXPERIMENT NO. 17.—On October 5 twelve young sweet-potato plants in pots were inoculated by inserting pycnospores and hyphæ of organism No. 102<sup>1</sup> (culture No. 2 of Sept. 25) into the lower part of the stem. Ten plants pricked with a sterile needle were left as checks.

Results.—On November 5 five plants, on November 11 one, on November 13 one, on November 15 one, on November 25 one, and on December 9 three, or a total of all 12 plants, were infected. None of the checks were infected. Pycnidia were found on ten of these plants when lifted and developed on the other two after three days in a moist chamber. The organism was recovered in pure cultures from seven plants. The experiment was terminated on December 9, 1913.

EXPERIMENT NO. 18.—On January 23 eight young sweet-potato plants in pots were inoculated by inserting pycnospores and hyphæ of organism No. 108<sup>2</sup> (culture No. 2 of Jan. 11) into the lower part of the stem. Six plants were left as checks.

Results.—On February 28 one plant, on March 8 two, on March 13 four, and on March 28 one, or a total of eight plants, were infected. Pycnidia were present on seven of the diseased plants when lifted and developed on the other one after 10 days in a moist chamber. None of the checks were diseased. Experiment terminated March 29, 1913.

EXPERIMENT NO. 19.—On February 19 ten young sweet-potato plants in pots were inoculated by inserting pycnospores and hyphæ of organism No. 108 (culture No. 3 of Jan. 11) into the stem. Seven plants were left as checks.

Results.—On March 21 four plants, on March 24 one, on March 31 one, on April 4 one, on April 18 one, and on April 26 one, or a total of nine plants, were infected.

<sup>1</sup> The organism recovered from plants inoculated in the greenhouse on Aug. 26 with No. 100 is known as No. 102.

<sup>2</sup> When the plants, inoculated on the Potomac Flats on Aug. 26, 1912, were dug, they were placed with the roots attached in moist chambers in the laboratory. After several weeks the fungus grew from the stem into the roots (Pl. XXIII, B), from which it was recovered. This organism was numbered "108."



Pycnidia were abundant on eight of the diseased plants when lifted on April 22. The one remaining diseased plant was lifted on April 26, and pycnidia were then present. The experiment was terminated April 26, 1913.

EXPERIMENT NO. 20.—On March 13 six young sweet-potato plants were inoculated by inserting spores and hyphæ into the lower part of the stem with organism No. 110<sup>1</sup> (culture No. 3 of Mar. 5). Five plants were left as checks.

Results.—On April 18 two plants, on April 22 two, and on April 23 one, or a total of five plants, were infected. The diseased plants were lifted on April 26, and pycnidia were present on all. None of the check plants were diseased. The experiment was terminated April 26, 1913.

#### INOCULATIONS IN THE LABORATORY

EXPERIMENT NO. 21.—On November 18 eight mature sweet potatoes (not plants) were inoculated by inserting pycnospores and hyphæ of organism No. 101 (culture No. 8 of Oct. 31) into the end of the potatoes. They were placed in cloth bags and stored in the laboratory. Four potatoes pricked with a sterile needle were used as checks.

Results.—No infection. The experiment was terminated January 31, 1913.

EXPERIMENT NO. 22.—On April 4, 1913, six sound sweet potatoes were prepared for inoculation by cutting away the ends of each so as to leave nothing but healthy tissue. They were then thoroughly washed and disinfected by treating with mercuric chlorid (1:1,000) for five minutes. They were afterwards rinsed in sterile water and placed in a moist chamber on filter paper disinfected with corrosive sublimate. Three of the potatoes were inoculated at the end and three at the side by inserting spores and hyphæ of organism No. 108 (culture No. 1 of Mar. 8). Four other potatoes pricked with a sterile needle were used as checks.

Results.—On April 15 no signs of decay had started at the point of inoculation. The filter paper appeared a little dry, and sterile water was added. After April 15 the rot developed and progressed rapidly in all the potatoes from the point of inoculation until by May 1 one potato was completely decayed and the others about one-third. Plate XXVIII, figure A, shows a sweet potato inoculated at the end and figure C, one inoculated at the side. Figures B and D are sections of figures A and C, respectively, showing the extent of the rot. The potatoes inoculated at the side decayed more rapidly than those inoculated at the end. Mature pycnidia and spores were formed on the surface on May 1. The organism was recovered from the pycnidia and from the diseased brown tissue of two potatoes.

The organism causes a chocolate-brown to almost black discoloration of the tissue, but leaves it rather firm, even in the later stages. This is not a distinctive characteristic, since there are a number of rots of the sweet potato, nearly all of which produce some shade of brown in the tissue and are in general so similar that it is practically impossible to separate them by their macroscopic appearances. All of the check potatoes remained sound.

<sup>1</sup> This organism was obtained from plants of *Ipomoea coccinea* which were inoculated with organism No. 101.

TABLE I.—Summary of results of inoculations with *Plenodomus destruens*.

Organism No.	Host.	Place of inoculation.	Method of inoculation.	Number—			Number of checks infected.	Experiment No. <sup>1</sup>
				In-oculated.	In-fected.	Checks.		
100...	<i>Ipomoea batatas</i> .	Potomac Flats.	By inserting spores and hyphæ into the lower part of the stem.	10	10	10	0	1
100...	do.	Greenhouse.	do.	10	8	5	0	2
101...	do.	do.	do.	10	10	6	0	3
101...	do.	do.	do.	10	10	6	0	7
100...	do.	do.	do.	8	6	6	0	9
100...	<i>Ipomoea purpurea</i> .	do.	do.	10	0	7	0	11
100...	<i>Ipomoea hederacea</i> .	do.	do.	10	0	7	0	12
101...	<i>Ipomoea coccinea</i> .	do.	do.	5	3	5	0	13
102...	do.	do.	do.	12	12	10	0	17
102...	do.	do.	do.	8	8	6	0	18
108...	do.	do.	do.	10	9	7	0	19
110...	do.	do.	do.	6	5	5	0	20
101...	do.	do.	By spraying foliage with spores suspended in water.	10	0	6	0	24
101...	do.	do.	do.	6	0	6	0	8
100...	do.	do.	do.	6	0	6	0	10
101...	do.	do.	By smearing conidia on lower part of stem.	10	7	6	0	5
101...	do.	do.	By pouring spores in water around the plant.	10	7	6	0	6
101...	<i>Ipomoea batatas</i> .	do.	By inserting hyphæ into the lower part of stem.	7	7	6	0	14
101...	do.	do.	By inserting spores and hyphæ into the node of vine several feet from the hill.	6	6	5	0	15
108...	do.	do.	do.	5	5	5	0	16
101...	Storage sweet potatoes.	Laboratory.	By inserting spores and hyphæ into the end of potato.	8	0	4	0	21
108...	do.	do.	do.	6	6	4	0	22

<sup>1</sup> For more complete data, the reader is referred to the experiments in the preceding pages corresponding to the numbers of this column.

<sup>2</sup> Experiments Nos. 4 to 7, inclusive, are combined in the body of the text, p. 258.

#### DISCUSSION OF INOCULATION EXPERIMENTS

Twenty-two sets of inoculations have been made with *Plenodomus destruens*, 17 of which were on sweet-potato plants. Eighty-four sweet-potato plants in nine different sets were inoculated by wounding the lower part of the stem and inserting spores and hyphæ. Seventy-eight died of the disease. Seven plants were wounded in a similar manner and inoculated with hyphæ only, and all became infected. Eleven vines in two sets were inoculated at the node several feet from the hill and 10 became diseased. Spores and hyphæ were smeared on the lower part of the stems of 10 plants, care being taken to cause no wounds, and 7 became diseased. Spores suspended in sterile water were poured about 10 plants, and 7 died from the organism. The foliage of 26 plants in

three different sets was sprayed with the spores suspended in water, but the disease was not produced thereby. Ten plants each of *Ipomoea hederacea* and *Ipomoea purpurea*, and 5 plants of *Ipomoea coccinea* were inoculated by inserting spores and hyphæ into the lower part of the stem. Three plants of *Ipomoea coccinea* were infected, the other species not being injured by the fungus.

Two sets of inoculations have been made with potatoes taken from storage. After inoculation one set was kept in the laboratory room in a cloth bag and gave negative results. In the other experiment the potatoes were placed in a damp chamber and kept moist with filter paper saturated with mercuric chlorid. Under these conditions the potatoes rotted readily. (Pl. XXVIII, figs. A, B, C, and D.) The organism was recovered in pure culture from the pycnidia formed thereon and from the rotten tissue within.

The results of these experiments show that the foot-rot organism is a vigorous wound parasite of *Ipomoea batatas*. In the greenhouse and in the field infection can be readily produced by wounding the plant, but this method is not imperative. It has been further shown that the temperatures and other environmental factors best suited for the growth of the plants are likewise most favorable for the development of the fungus. During warm, moist weather, when the plants grow most vigorously, the disease was more severe than when growth was retarded by low temperature. Plants at all ages were about equally susceptible to the disease.

It is also interesting to note in this connection that infection was readily produced by inoculating with hyphæ only, the result showing that the progress of the disease was more rapid and the plants killed sooner than when inoculations were made with spores.

#### HOW THE DISEASE IS PERPETUATED

The exact life history of this fungus will be in doubt so long as a perfect stage is not known. It is evident, however, that an ascogenous stage is not necessary to carry it from one season to the next. Diseased specimens on which there were numerous pycnidia were wintered out in a wire cage covered over with leaves and some dirt with the hope that an ascospore stage might develop. On the 27th of the following April the specimens were examined, and normal pycnospores but no asci were found.

A second lot of diseased specimens were wintered out in a wire cage set on the ledge of a north window, where they were subjected to alternately dry and wet weather and other atmospheric changes. When these were examined on May 20, 1913, numerous normal conidia were present and the organism recovered in culture.

There are at least two ways by means of which this disease may be carried from one year to another: (1) On the dead vines and (2) on the

potatoes in storage. In the locality in which this disease occurs, the hotbeds are started about April 1, or even sooner, so that infection of young plants might easily take place from pycnospores that had endured as late as May 20. In old fields the beds are often made from the soil on which sweet potatoes have been grown the previous year, thereby providing the best conditions possible for direct infection of the new crop. Furthermore, it was previously pointed out that the foot-rot organism spreads from diseased stems to the potatoes and develops pycnidia thereon. Experiments have also shown that under hotbed conditions the organism will grow from diseased potatoes on to the slips produced therefrom. Therefore, owing to the comparative obscurity of diseases of this type, infected roots might readily be overlooked when selecting seed, thereby making the sprouts growing from such potatoes liable to infection.

The brown, spherical, thick-walled, chlamydosporelike bodies were found in abundance embedded in the cortex of diseased parts of plants wintered out in the wire cages. What function these forms have is not yet known, although it is possible that they are able to reproduce the fungus and serve to carry the organism through unfavorable conditions. Repeated attempts, however, to germinate them have always given negative results.

#### SOME PHYSIOLOGICAL CHARACTERISTICS OF THE FUNGUS

##### CHARACTER OF GROWTH ON DIFFERENT CULTURE MEDIA

The foot-rot fungus grows well on some kinds of media, but sparsely on others. The growth on some media may be regarded as characteristic of the organism and is unlike that of any other fungus with which the writer is familiar.

A comparative study of growth has been made on nine different culture media—i. e., corn meal, string-bean agar, string beans, Irish-potato cylinders, sweet-potato cylinders, sweet-potato stems, rice, beef bouillon, and beef agar. These different media have not been selected for any particular reason, except that they are those commonly used and can easily be duplicated. Five tubes (flasks in case of corn meal) were inoculated on November 25, 1912, with conidia from a 25-day-old culture grown on corn meal. The tubes and flasks were kept in the light on a table in the laboratory, the temperature of which varied from 18° to 24° C. They were kept under observation until January 31, 1913, after which, owing to the dried condition of the cultures, no more notes were made. The following records, given in number of days from the beginning of the experiment, show the nature of the growth on the different media.

CORN MEAL (1061<sup>1</sup>)

- 2 days.—No visible growth.
- 4 days.—Yellowish white growth about 1 cm. in diameter. Hyphæ growing close to the medium.
- 7 days.—Hyphal growth about 4 cm. in diameter, slightly yellowish. Numerous minute black pycnidia covering an area of about 2 cm. in diameter in the center of the growth.
- 9 days.—Hyphal growth covering most of the surface of the medium and pycnidia formed over about two-thirds. Spores just beginning to exude from pycnidia.
- 11 days.—Pycnidia covering most of surface of medium; exudate of spores forming small viscid droplets.
- 14 to 17 days.—Abundant discharge of spores from the pycnidia.
- 21 days.—Spore discharge collecting in large globules, forming an almost continuous covering over the surface of the medium.
- 25 days.—No change.
- 40 days.—Surface of medium completely covered with a slimy liquid containing pycnospores.
- 67 days.—Hyphæ hyaline. Numerous intercellular and terminal chlamydosporelike bodies.

Corn meal is the best of the media used for the development of pycnidia. The pycnospores are first expelled in about one week, the process continuing for 30 or 40 days thereafter. At the end of that time the medium is covered with a slimy liquid in which the spores are suspended. This liquid, often amounting to 5 c. c., is characteristic of growth on this medium and is apparently not due to the water added, since that is taken up by the corn meal.

## STRING-BEAN AGAR (1037)

- 4 days.—Sparse white growth.
- 7 days.—Heavy, white flaky growth of erect hyphæ covering one-fourth of slant.
- 9 days.—Light-colored pycnidia collected in spots on surface of medium.
- 11 days.—No increase in mycelial growth; pycnidia dark; pycnospores exuding from pycnidia.
- 14 to 17 days.—Perceptible increase in the exudation of pycnospores.
- 21 days.—Exudate colorless, forming large droplets and uniting.
- 25 days.—No apparent change.
- 40 days.—Hyphal growth covering most of slant. Spores normal.
- 67 days.—Hyphæ hyaline. A few chlamydosporelike bodies.

String-bean agar is only a fair medium for the growth of this fungus. The pycnidia were sparingly formed as compared with the growth on corn meal.

## STRING BEANS (1063)

- 4 days.—White, loose, flaky growth covering one-third of medium.
- 7 days.—White, loose, flaky growth covering three-fourths of medium.
- 9 days.—Feltly grayish white growth of somewhat erect hyphæ. Pycnidia collected in spots. Pycnospores present.
- 11 days.—Pycnidia black.
- 14 days.—Slight exudate of spores from pycnidia.
- 17 days.—Slight increase in the discharge of spores.
- 21 to 25 days.—Exudates uniting, colorless.

<sup>1</sup> A number is given to and a description made of each medium when it is prepared in the laboratory so that it can be readily duplicated when desired. Unless otherwise stated, all media were prepared in the laboratory of the Office of Cotton and Truck Disease and Sugar-Plant Investigations.

- 40 days.—Medium studded with pycnidia. Exudate abundant. Pycnospores not typical, being immature in appearance and irregular in shape.  
67 days.—Hyphæ hyaline. Many chlamydosporelike bodies. Long cylindrical bodies present. (Pl. XXV, G.)

## IRISH-POTATO CYLINDERS (1036)

- 4 days.—Dense, felty white growth covering all of potato cylinder. Medium slightly darkened.  
7 days.—Scattered dark (not black) pycnidia forming.  
9 days.—Pycnidia abundant, irregularly scattered; black, rather large.  
11 days.—Pycnidia black and conspicuous; uniformly scattered over the medium.  
14 days.—A slight exudate of spores from pycnidia.  
17 days.—Pycnidia crowded together. Slight discharge of spores.  
21 to 25 days.—Pycnidia numerous. No discharge of spores from the pycnidia.  
40 days.—Potato cylinder studded with pycnidia. No discharge of spores. Pycnospores abnormal, being apparently immature and irregular in shape.  
67 days.—Hyphæ hyaline. A few chlamydosporelike bodies. Long cylindrical bodies present. (Pl. XXV, G.)

## SWEET-POTATO CYLINDERS (1064)

- 4 days.—White procumbent growth of fairly dense hyphæ covering one-half of potato cylinder. Medium changed to a light chocolate-brown color.  
7 days.—Feltlike growth covering all of medium. Potato cylinders changed to a chocolate-brown color. Pycnidia forming; surface of medium grayish.  
9 days.—Pycnidia crowded together, forming a felty grayish surface on the medium.  
11 days.—Pycnidia formed in a dense grayish mass over surface of medium. Spores exuding from the pycnidia.  
14 to 25 days.—Slight discharge of spores.  
40 days.—Medium covered with pycnidia. Spores exuding abundantly from one tube, a little from another, and none from the remaining tubes.  
67 days.—Many long cylindrical bodies. Hyphæ hyaline.

## SWEET-POTATO STEMS (1049)

- 4 days.—A sparse spreading growth of white hyphæ covering one-fourth of stem.  
7 days.—Sparse, grayish, somewhat irregular, cottony growth of erect hyphæ. Pycnidia black, larger than on corn meal, and resembling those on the vines under natural conditions.  
9 days.—Pycnidia black, uniformly distributed over medium. Spores exuding from the pycnidia.  
11 days.—Pycnidia numerous; pycnospores exuding from pycnidia in brownish globules.  
14 to 17 days.—Increase in the discharge of spores from the pycnidia.  
21 to 25 days.—Exudates from the pycnidia uniting.  
40 days.—Stems studded with pycnidia with long beaks. Discharge of spores from the pycnidia less than on corn meal.  
67 days.—Hyphæ somewhat brown. A few long cylindrical bodies

## RICE (967)

- 4 days.—No visible growth.  
7 days.—Very slight mycelial growth. Many black, somewhat large pycnidia.  
9 days.—Mycelial growth sparse. Spores just beginning to ooze from the pycnidia.  
11 days.—Surface of medium studded with black pycnidia. Spores discharged from the pycnidia in small globules.

- 14 to 17 days.—Discharge of spores from the pycnidia increasing.  
 21 to 25 days.—Exudates from the pycnidia increasing and uniting.  
 40 days.—Pycnidia abundant, forming a black crust over the surface of the medium.  
 Exudate of spores from the pycnidia a yellowish slimy mass.  
 67 days.—A few long cylindrical bodies present. Hyphæ mostly hyaline. No chlamydosporelike bodies.

Rice, with the exception of corn meal, is the best of all the media tried. Spores are formed abundantly and exuded in large droplets from the pycnidia. A very scant mycelial growth is formed on rice.

#### BOUILLON (5725<sup>1</sup>)

- 3 days.—No visible growth.  
 6 days.—A very sparse flaky white growth arising from individual spores lodged against the glass below the surface of the medium.  
 8 days.—Growth below the surface of the medium from individual spores enlarging and adhering to the glass. No floating hyphæ. Slight surface growth against the glass.  
 10 days.—Increase in mycelial growth.  
 14 days.—Pycnidia forming a black ring against the glass at the surface of the medium.  
 29 days.—Pycnospores very few, poorly developed, and not typical of the spores on corn meal or rice.  
 56 days.—Hyphæ hyaline, with many intercellular, spherical swellings singly or in chains.

#### BEEF AGAR (5726<sup>1</sup>)

- 3 days.—Grayish, thick, felty growth, extending  $\frac{1}{4}$  cm. above surface of medium; irregular in outline.  
 6 days.—Growth spreading; white pycnidia forming.  
 8–10 days.—Pycnidia forming a black line on the surface of the medium at the point of contact with the glass; elsewhere on the surface of medium they are collected into spots.  
 14 days.—Pycnidia large and black and increasing in number.  
 29 days.—No pycnidia at the edge of mycelial growth except in contact with glass. Spores few and imperfectly formed.  
 56 days.—Hyphæ hyaline, with many intercellularly or terminal spherical bodies several times the diameter of the hyphæ arranged singly or in chains. Very few typical spores.

These results of tests with the different media<sup>2</sup> bring out clearly the fact that the development of the foot-rot organism is decidedly good on some media and very poor on others. Numerous pycnidia and an

<sup>1</sup> This medium was prepared in the Laboratory of Plant Pathology.

<sup>2</sup> In addition to the results of growth obtained on the nine media discussed in the preceding pages, the fungus was cultivated on a number of others, but not for the purpose of comparing the growth at the end of stated periods of time; hence, these have not been included in the general description. Growth of the fungus has been studied on mature stems of cotton (*Gossypium herbaceum*), sweet clover (*Melilotus alba*), also on immature stems of sweet clover, lupine (*Lupinus* sp.; common varieties from Germany), oak (*Quercus* sp.), tomato (*Lycopersicon esculentum*), and sweet potato (*Ipomoea batatas*). The growth and production of fruiting bodies varied greatly on the different media. On oak and cotton there was but a sparse growth, although a few pycnidia were formed. On tomato there was practically no growth. Numerous fruiting bodies were produced on stems of sweet clover (mature and immature), sweet potato, and a fair growth of hyphæ with production of pycnidia on lupine. Mycelium is so sparingly formed that when produced abundantly it is a sign that the medium is not suited to the best development of the fungus. The production of pycnidia, on the other hand, is evidence that the medium is a most favorable one.

abundance of pycnospores are produced on corn meal and rice. On beef agar and bouillon, on the other hand, the pycnidia are comparatively few and mostly sterile. The development of pycnidia on steamed sweet-potato stems was very similar to that found in nature, except that the beaks were longer. A fair growth only was made on string beans and Irish-potato and sweet-potato cylinders. Corn meal and rice, however, are the only media tried on which the growth could be regarded as showing typical development, it being quite apparent in most other cases that the conditions were quite abnormal.

#### GERMINATION OF PYCNOSPORES

Germination begins in about  $6\frac{1}{2}$  to 7 hours in hanging drop cultures. Growth in sterile or hydrant water is slow at first, the germ tube reaching only about one-half to one and one-half times the length of the spore in 24 hours at room temperature ( $21.5^{\circ}$  to  $22.5^{\circ}$  C.). In nutrient media a much better growth is made. At the end of 48 hours the germ tube reaches a length several times that of the spore and begins branchings. (Pl. XXV, H.) Growth is fairly rapid thereafter, although if compared with certain *Fusaria* it would be regarded as a slow-growing organism both in artificial culture and on the host. Preliminary to germination, the pycnospores swell up, lose their original shape, and become more nearly spherical.

#### INFLUENCE OF TEMPERATURE ON THE GERMINATION OF PYCNOSPORES

The minimum, optimum, and maximum temperatures for germination of the pycnospores were determined by inoculating about 2 c. c. of rice water in test tubes with spores of the fungus from a young culture on corn meal. One set of cultures was placed in each of six thermostats, the range of temperature being indicated in Table II. Another set was similarly inoculated and placed in the laboratory room as a check. The cultures were examined at the end of 18 hours, and those that had germinated freely were thrown out. The other cultures were continued for 24, 42, or 48 hours, as necessity required.

TABLE II.—*Limiting temperature for the germination of pycnospores.*

Thermo- stat.	Temper- ature.	Time in hours.			
		18	24	42	48
	$^{\circ}$ C.				
VI.....	11.5 to 12.0	No germination.....		Slight germination.....	
VIII....	15.0 to 17.0	Slight germination.....		Fair germination.....	
Room ..	21.0 to 22.0	Fair germination.....			
X.....	25.0 to 26.0	Good germination.....			
XII....	35.5 to 36.0	do.....			
XIII...	37.4 to 37.5	Fair germination.....	Fair germination.....		
XIV....	40.0 to 40.5				No germination.



While no absolute limits have been established, Table II shows that only a very slight germination of spores took place in thermostat VI ( $11.5^{\circ}$  to  $12^{\circ}$  C.) at the end of 24 hours. As the temperature was increased, germination became better until the optimum was reached in thermostats X ( $25.0^{\circ}$  to  $26.0^{\circ}$  C.) and XII ( $35.5^{\circ}$  to  $36.0^{\circ}$  C.). Germination was somewhat reduced in thermostat XIII ( $37.4^{\circ}$  to  $37.5^{\circ}$  C.) and completely prohibited in thermostat XIV ( $40.0^{\circ}$  to  $40.5^{\circ}$  C.). Of the temperatures tried the minimum for germination would be found in thermostat VI ( $11.5^{\circ}$  to  $12.0^{\circ}$  C.), the optimum in thermostats X ( $25.0^{\circ}$  to  $26.0^{\circ}$  C.) and XII ( $35.5^{\circ}$  to  $36.0^{\circ}$  C.), and the maximum in thermostat XIV ( $40.0^{\circ}$  to  $40.5^{\circ}$  C.).

#### VIABILITY OF PYCNOSPORES

Just how long the spores will retain their viability in a dried condition is not known. The pycnospores on material collected on August 22, 1912, and kept in an envelope in the laboratory would not germinate in plates of beef agar made on November 27. Hanging-drop cultures were made with hydrant water in Van Tieghem cells from the same material on December 11, with similarly negative results. On the other hand, pure cultures made on August 15, 1912, on corn meal retained their viability to June 18, 1913. These results are not directly comparable, since there is always a certain amount of moisture present in the medium when the culture is started. Furthermore, as was previously pointed out, this organism produces a considerable amount of liquid on corn meal, even though there is no surplus water present in the culture when inoculated.

#### INFLUENCE OF TEMPERATURE ON GROWTH

The influence of temperature on the growth of *Plenodomus destruens* in cultures was determined by the use of 10 thermostats ranging in average temperatures from  $1.09^{\circ}$  to  $37.3^{\circ}$  C., and in the laboratory with an average temperature of  $21.9^{\circ}$  C. These temperatures varied somewhat, as will be seen by referring to Table III, where the average maximum and minimum temperature for each thermostat is recorded.

Cultures were made on January 15, 1913, on sterilized rice (1085) in test tubes, it having been previously ascertained that this was a favorable medium for the growth of the fungus. Five tubes were placed in each of the 10 thermostats and one set in the culture room in the laboratory as a check. The cultures were kept in the incubators and under observation for 21 days. Table III contains a record of the growth of the organism in each thermostat and in the laboratory room on the different dates covered by the experiment.

TABLE III.—Record of growth in laboratory room and in 10 thermostats maintained at different temperatures (°C.).<sup>1</sup>

Date.	I. Average, 1.59; maximum, 1.66; minimum, 0.6°.	II. Average, 4.5; maximum, 5.7; minimum, 3.2°.	III. Average, 7.6; maximum, 9.0; minimum, 6.4°.	V. Average, 9.0; maximum, 10.0; minimum, 8.0°.	VI. Average, 12.6; maximum, 14.0; minimum, 11.2°.	VII. Average, 15.2; maximum, 16.7; minimum, 13.9°.	IX. Average, 16.4°; maximum, 18.5°; minimum, 15.3°.	X. Average, 17.6°; maximum, 19.5°; minimum, 16.3°.	Room. <sup>2</sup> Average, 21.9°; maximum, 24.5°; minimum, 17.2°.	XI. Average, 30.2°; maximum, 38.0°; minimum, 29.4°.	XII. Average, 37.3°; maximum, 38.0°; minimum, 36.4°.
Jan. 20.....	No growth	No growth	No growth	No growth	No growth	No growth	No growth....	No growth....	Sparse hyphal growth.	Some mycelial growth; a few scattered pycnidia.	Very slight growth.
Jan. 21.....	do.	do.	do.	do.	do.	do.	do.	do.	Increased hyphal growth. Some pycnidia formed.	Increase in hyphal growth and number of pycnidia.	No change
Jan. 23.....	do.	do.	do.	do.	do.	do.	do.	do.	Continued good growth and increase in number of pycnidia.	Increase in hyphal growth and number of pycnidia. Yellow discoloration of medium.	Do.
Jan. 24.....	do.	do.	do.	do.	do.	do.	Scant growth of hyphae; very few pycnidia.	Scant growth of hyphae; very few pycnidia.	Pycnidia numerous.	Abundant hyphal growth. Pycnidia fewer than at room temperature.	Do.
Jan. 27.....	do.	do.	do.	do.	do.	Few pycnidia forming.	Pycnidia fewer than in thermostat X.	Pycnidia many	Pycnidia numerous; spores exuding in small droplets.	Pycnidia numerous; spores exuding in small droplets; medium yellowed.	Do.
Jan. 30.....	do.	do.	do.	do.	do.	Pycnidia few	Pycnidia many; spores exuding slightly.	Pycnidia many; spores exuding slightly.	do.	Hyphal growth better than at any other temperature.	Do.
Feb. 8.....	do.	do.	do.	do.	do.	Pycnidia few; slight discharge of spores.	Hyphal growth less than in thermostat X; spores exuding.	Fair hyphal growth; spores exuding.	Best growth, formation of pycnidia, and discharge of spores at this temperature.	Spore discharge yellowish.	Do.
Feb. 6.....	do.	do.	do.	do.	do.	A fair growth of hyphae; pycnidia fairly abundant; spores exuding.	Pycnidia very abundant; spores exuding.	Pycnidia very abundant; spores exuding.	Pycnidia very numerous; spores discharged more abundantly than at any other temperature.	Spores atypical; no further discharge of pycnidia.	Do.

<sup>1</sup> Temperature readings were made about 9:15 a. m. and 4:15 p. m. each day.<sup>2</sup> The cultures were kept in a culture room in the middle of the laboratory.

It is seen from Table III and also from figure 2 that the temperatures of thermostats I, II, III, and V ( $1.09^{\circ}$  to  $9.0^{\circ}$  C.) are prohibitive of growth. A sparse growth took place in thermostat VI ( $11.2^{\circ}$  to  $14.0^{\circ}$  C.) and reached its maximum growth in the laboratory room ( $17.2^{\circ}$  to  $24.5^{\circ}$  C.). The best growth was obtained at an average temperature of  $21.9^{\circ}$  C. and the next best in thermostat XI ( $29.4^{\circ}$  to  $30.8^{\circ}$  C.). The growth of mycelia in thermostat XI ( $29.4^{\circ}$  to  $30.8^{\circ}$  C.) was better at the outset than at any other temperature, although the production of pycnidia and spores was not as good at the end of the experiment as in cultures growing in the laboratory room. The medium in thermostat XI was decidedly discolored, a change which did not occur at any other temperature. In

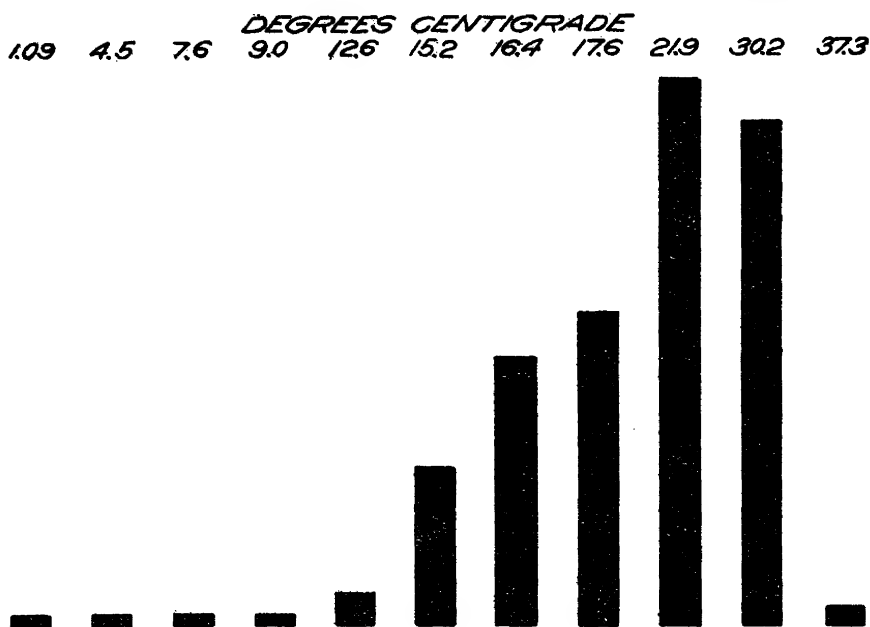


FIG. 1.—Graphic representation of growth on rice at different temperatures.

thermostat XII ( $36.4^{\circ}$  to  $38.0^{\circ}$  C.) a very slight growth of hyphae took place during the first three or four days. No further development took place thereafter. While these results do not definitely fix the limiting temperature for growth, they show that the optimum probably lies somewhere between  $21.9^{\circ}$  and  $30.2^{\circ}$  C., the minimum close to  $12.6^{\circ}$  C., and the maximum at about  $37.3^{\circ}$  C.

At the conclusion of the incubation period all the cultures were taken from the thermostats and placed on a table in the laboratory room. At the end of 10 days there was a good growth in all the tubes except in those that were in thermostat XII ( $36.4^{\circ}$  to  $38.0^{\circ}$  C.), cultures of which had been killed in 21 days.

A comparison of these results with similar experiments carried on with the pycnidial stage of *Diaporthe batatatis*<sup>1</sup> shows that *Plenodomus destruens* is limited to a narrower range of temperatures in its growth in artificial cultures. The optimum temperature for growth of the dry-rot organism was 3° C. higher than that of the foot-rot fungus. At the lower temperatures the former made as good a growth at an average temperature of 7.5° C. as the latter did at an average temperature of 12.6° C. At the higher temperature the foot-rot fungus was killed when exposed for 21 days at an average temperature of 37.3° C., while the dry-rot organism made some growth when exposed for 18 days at an average temperature of 37.8° C.

#### INFLUENCE OF LIGHT ON THE GROWTH AND PRODUCTION OF PYCNIDIA

It was found that the conidial stage of the dry-rot fungus (*Diaporthe batatatis*) produced pycnidia only sparingly unless exposed to light.<sup>1</sup> Contrary to these results, the foot-rot organism on rice cultures grew equally well in darkness and in the light. Pycnidia were formed in about 3 days, and the spores began exuding in small droplets in about 10 days.

#### DISSEMINATION OF THE DISEASE

From what we already know of the foot-rot disease it is evident that there are several ways in which the organism may be carried from one place to another. In view of the fact that the pycnospores will live through the winter on the dead vines until as late as May 20, the plants in near-by hotbeds and even in the fields are liable to infection from this source. It has been shown that the organism causes a serious disease of the stem of the plant and grows from there to the roots, forming pycnidia on the surface. It is evident, therefore, that the use of such potatoes for seed might account for a large part of the infections.

There is no way of determining to what extent insects, the wind, and such agencies are responsible for the distribution of the disease, but they, do doubt, play an important part. It is believed that this disease, like many others, is also distributed from one field to another on farm implements, the hoofs of animals, or by means of stable manure, etc. It is a well-known fact that many farmers are careless about the disposition of diseased and decayed sweet potatoes. Without suspecting the risk they are taking, they often throw them on the manure pile or feed them to stock without cooking. In either case the organism, if present on the potatoes, might eventually be carried to the field. The wider distribution of the disease—i. e., from one locality to another—must largely be accounted for by the exchange of seed potatoes and seed plants.

<sup>1</sup> Harter, L. L., and Field, Ethel C. A dry rot of sweet potatoes caused by *Diaporthe batatatis*. U. S. Dept. Agr., Bur. Plant Indus., Bul. 281, 38 p., 4 pl., 1913.

## POSSIBLE METHODS OF CONTROL

The suggestions here given for the control of foot-rot are not based on experimental evidence, but on what would seem obvious from a knowledge of the disease and the methods of handling the crop. It has already been pointed out (1) that the disease occurs both on the stem and roots of sweet-potato plants; and (2) that the pycnospores of the fungus can live through the winter and late enough the following spring to infect the new crop. With these facts in mind it will be clear that precautionary and sanitary measures should be employed. One of these should consist in the careful selection of healthy potatoes for seed. Selection should be made preferably in the fall at digging time and any suspicious potatoes should be discarded. They should be carefully examined again in the spring when the disease is more easily recognized, and all those that show any sign of disease should be rejected. While disinfection of the seed in a solution of mercuric chlorid (1:1,000) will not destroy the fungus buried beneath the surface of the potato, it will kill all adhering spores and clean the potatoes so that diseased spots can be more readily detected. After immersing for five minutes in the solution, the potatoes should be rinsed in water and thoroughly dried. It is advisable that disinfection be done on a clear, warm day, just before the potatoes are put in the bed.

Soil that is likely to be infected with the disease should not be used in the preparation of the hotbed. If, however, disease-free soil can not be obtained, then it should be disinfected by steaming for one hour at a temperature of 100° C. If steam sterilization is not feasible, the soil may be soaked in a formaldehyde (40 per cent) solution (1:200). If the latter method of disinfection is employed, the soil should be treated at least 10 days before it is to be used, and it should be occasionally stirred to assist in the escape of the gas.

All decayed, diseased, or discarded potatoes should not be fed raw to stock, or thrown on the manure pile to compost, but should be cooked; neither should the potatoes be thrown on the ground around the hotbed. These practices are too common, and are liable to infect otherwise disease-free beds.

Crop rotation is a good practice, whether for the control of diseases or not, and should be practiced by every farmer. It is not yet known how long this disease retains its vitality in the soil without sweet potatoes as a host, but probably for several years. At least three years should be allowed between crops whenever diseases of this type are found, although it is doubtful if this length of time will completely eradicate it from the soil, but it should reduce it considerably.

## SUMMARY

(1) The foot-rot has been hitherto unknown on the sweet potato (*Ipomoea batatas*). It is caused by the fungus *Plenodomus destruens*.

(2) The organism is a very destructive wound parasite of the sweet potato in the vicinity of the Dismal Swamp, Va., and occurs at Cape Charles and Keller, Va.

(3) It kills the plant by the destruction of the cortex of the stem near the ground.

(4) Pycnidia are abundantly formed on the diseased area of the stem about the time the plant dies, or soon thereafter.

(5) The disease, while primarily found on the stem, invades the roots and vines also.

(6) The fungus is cultivable on most artificial media, but gives the highest development on corn meal, rice, and stems of the sweet potato.

(7) The parasitism of the organism has been proved by numerous inoculations of plants grown on the Potomac Flats and in the greenhouse.

(8) Successful infection experiments were carried out with reisolations of the fungus from inoculated plants.

(9) The organism is parasitic on *Ipomoea coccinea*, but not on *I. purpurea* and *I. hederacea*.

(10) Sweet potatoes from storage are decayed by the fungus when inoculated under sterile conditions and kept moist in light.

(11) Light has no apparent effect on the production of fruiting bodies in pure cultures of rice.

(12) The fungus makes its best growth, as measured by abundance and rapidity of sporulation, in rice cultures at an average temperature of about 21.9° C.

(13) The fungus can live through the winter on dead vines of the sweet potato.

(14) The disease is probably disseminated principally by means of "seed roots" and the slips produced therefrom.

(15) Seed beds should be sterilized, and potatoes to be used for seed should be carefully selected.

## DESCRIPTION OF PLATES

- PLATE XXIII. Parts of sweet-potato plants, showing the presence of pycnidia: *A*, On the stem just above the ground; *B*, on the root.
- XXIV. Portion of sweet-potato vines several feet from the hill, showing the results of a natural infection of the foot-rot fungus. The organism was recovered from these vines before being photographed.
- XXV. Microscopic characters of the foot-rot fungus: *A*, Section through a pycnidium on the root; *B*, section through a pycnidium on the stem; *C*, hyphae, from artificial culture; *D* and *E*, chlamydospore-like bodies found on the host and in some culture media; *F*, pycnospores; *G*, club-shaped bodies often found in pycnidia; *H*, germinating pycnospores.
- XXVI. Two sweet-potato plants in pots, demonstrating the parasitism of the foot-rot fungus: *A*, Inoculated; *B*, not inoculated.
- XXVII. Nine-day-old cultures on synthetic agar: *A*, The conidial stage of *Diaporthe batatatis*; *B*, *Plenodomus destruens*.
- XXVIII. Sweet potatoes inoculated with *Plenodomus destruens*: *A*, Inoculated at the end; *B*, a section of *A* showing extent of rot; *C*, inoculated at the side; *D*, section of *C* showing the extent of rot.

(274)

---

ADDITIONAL COPIES of this publication  
may be procured from the SUPERINTEND-  
ENT OF DOCUMENTS, Government Printing  
Office, Washington, D. C., at 25 cents per copy

---

Subscription price per year - - - - \$2.50

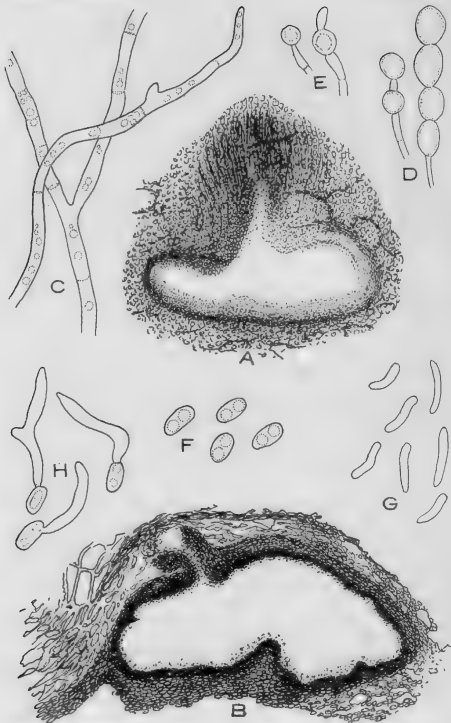
---



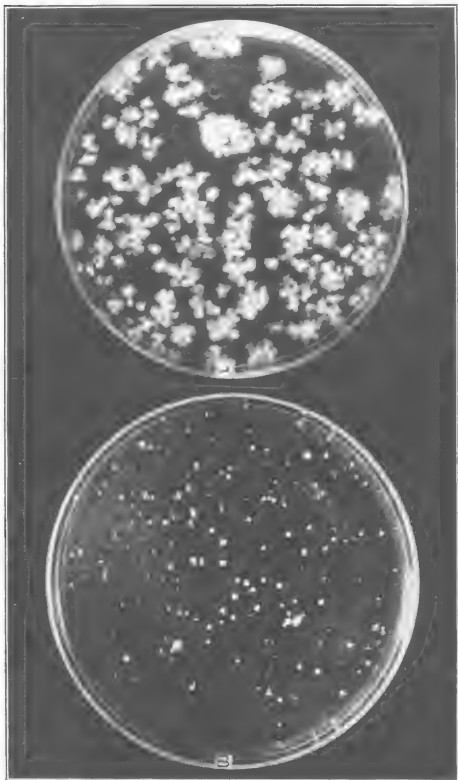














# JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. I

WASHINGTON, D. C., JANUARY 10, 1914

No. 4

## ENVIRONMENTAL INFLUENCES ON THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF WHEAT

By J. A. LE CLERC, *Chief*, and P. A. YODER, *Assistant Chemist*,  
*Plant-Chemistry Laboratory, Bureau of Chemistry*

### INTRODUCTION

A former series of experiments <sup>1</sup> conducted in the Bureau of Chemistry showed that neither the composition nor the physical characteristics of wheat are to any great extent hereditary. The protein, gluten, and ash contents, as well as the size of the berry, the weight of a bushel, and the flintiness of the kernel, were found to be dependent upon the climatic conditions prevailing during the growing period of the plant. Seed of Kansas wheat containing 20 per cent of protein and showing 100 per cent of flinty kernels and seed of California wheat containing 10 per cent of protein with 13 per cent of flinty kernels when grown side by side in South Dakota yielded crops of identical composition and physical appearance. The same was true of these Kansas and California seeds when grown in California. The crops grown in California were, however, entirely unlike those grown in South Dakota, owing to the great difference in climatic conditions. It was shown in a most conclusive manner that environment plays a major part in influencing both the chemical composition and the physical appearance of a wheat crop. Cropping through a number of generations under widely different environments therefore does not alter permanently or make a noticeable impression upon the transmissible physical and chemical properties of wheat.

Similar experiments, involving the transference of soil, are reported by Shaw and Walters.<sup>2</sup> In the main, their observations, based on crops grown throughout a period of three years in one locality, harmonize with the conclusions here presented, which are founded on the wider range of experimental data now at hand, involving crops grown for four years on three different types of soil in three different localities having widely

<sup>1</sup> Le Clerc, J. A., and Leavitt, Sherman. Tri-local experiments on the influence of environment on the composition of wheat. U. S. Dept. Agr., Bur. Chem. Bul. 128, 18 p., 1910.

<sup>2</sup> Shaw, G. W., and Walters, E. H. A progress report upon soil and climatic factors influencing the composition of wheat. Cal. Agr. Exp. Sta. Bul. 216, p. 549-574, 1911.

varying climatic conditions. In some particulars, however, the conclusions which seemed justifiable from their experiments are not borne out by these more extensive data.

The experiments discussed in this article were designed to study further the environmental influences and to show the rôle exerted by the soil and the part played by climatic conditions, such as rainfall, sunshine, humidity of the atmosphere, temperature, winds, and elevation above sea level. As in the case of the previous experiments,<sup>1</sup> they were carried on in cooperation with the Office of Cereal Investigations of the Bureau of Plant Industry. The agricultural experiment stations of Maryland, Kansas, and California cooperated by growing the crops.

#### CONDUCT OF THE EXPERIMENTS

In order to distinguish between the rôle played by soil and that by environment other than soil, samples of soil were interchanged among three localities, Maryland (College Park), Kansas (Hays), and California (Davis), which differ widely in climatic conditions. From each locality sections of a normally fertile wheat-producing soil 5 feet square and 3 feet deep were dug up in 3-inch layers, sacked, and replaced in the same original position. To obviate any differences due to this manipulation a portion of soil 5 feet square and 3 feet deep from each locality was likewise dug up in 3-inch layers, sacked, and stored until the soils from the two other localities had arrived, when all three samples were placed in their respective positions. A fourth plat of soil of the same size was allowed to remain undisturbed in each locality to determine whether the treatment to which the three other soils had been subjected would exert any influence on the composition of the grain. Thus, there were 12 experimental plats, 4 in each locality, as shown in the following plan:

##### TWELVE EXPERIMENTAL PLATS

###### California:

Plat of undisturbed California soil, or check plat.	
Plat of disturbed California soil.	} Each taken up in 3-inch layers and replaced in original order.
Plat of Kansas soil.	
Plat of Maryland soil.	

###### Kansas:

Plat of undisturbed Kansas soil, or check plat.	
Plat of disturbed Kansas soil.	} Each taken up in 3-inch layers and replaced in original order.
Plat of California soil.	
Plat of Maryland soil.	

###### Maryland:

Plat of undisturbed Maryland soil, or check plat.	
Plat of disturbed Maryland soil.	} Each taken up in 3-inch layers and replaced in original order.
Plat of California soil.	
Plat of Kansas soil.	

---

<sup>1</sup> Le Clerc and Leavitt. *Op. cit.*

During the first two years, 1908 and 1909, Crimean wheat obtained from seed grown in Kansas was used on all 12 plats. As this variety was not adapted to conditions prevailing in Maryland and California, Turkey wheat was selected for 1910, 1911, and 1912. The change from Crimean to Turkey wheat did not interfere, however, with the object of the experiment, which was to determine the influence exerted by climatic conditions and soil on the composition of the crop.

The following determinations were made according to the methods given in Bulletin 107, Revised, of the Bureau of Chemistry, entitled "Official and Provisional Methods of Analysis."

Water; weight of 1,000 grains; weight of a bushel; flinty grains; nitrogen; alcohol-soluble nitrogen; fat; fiber; pentosans; sugars; ash; phosphoric acid; and potash. The alcohol-soluble nitrogen was determined by treating a certain quantity of ground wheat with a 70 per cent solution of alcohol at ordinary temperature, with frequent shaking, for several hours, and then allowing the solution to stand overnight. An aliquot part was taken and the nitrogen therein determined. The amount of nitrogen thus obtained divided by the total quantity of nitrogen in the sample gave the gliadin number.

#### TABULATION OF DATA

The data are collected in a number of tables. In Table I, first column, is given the analysis of the original seed grown in Kansas in 1908, which was used as seed on all the plats for the following year's crop. The other analyses in Table I and the data in Tables II to IV were obtained on crops grown in 1909, 1910, 1911, and 1912, the results being grouped by locality. The data from the different soil plats and the check-soil plat in each locality are arranged in adjacent columns in Table I. In Table II the same data, exclusive of check-plat data, are rearranged, the results from the same soils being grouped in adjacent columns. Averages derived from these data are given in Tables III, IV, and V. In Table III are shown the averages of all the constituents from crops grown in California, Kansas, and Maryland, not including the check-soil plat, throughout the four years of the experiment. Table IV gives the averages obtained from data on the crops grown on the soils of California, Kansas, and Maryland for each of the three localities and for all four years. Finally, in Table V are shown the averages for the undisturbed or check-soil plats and for the corresponding plats in which the soil had been taken up in 3-inch layers and replaced.



TABLE I.—Composition of wheat grown on different plats of soil in California, in Kansas, and in Maryland in 1909, 1910, 1911, and 1912.

## CRIMEAN WHEAT.

Original seed and 1909 crop.

Determination.	Original seed grown in Kansas in 1908.	Wheat grown in California on—				Wheat grown in Kansas <sup>1</sup> on—				Wheat grown in Maryland on—			
		California check soil.	California soil.	Kansas soil.	Maryland soil.	California soil.	Kansas soil.	Kansas check soil.	Maryland soil.	California soil.	Kansas soil.	Maryland soil.	Maryland check soil.
Physical properties:													
Water.....per cent..	9.20	9.64	8.98	9.00	8.88	.....	.....	.....	.....	9.56	9.48	9.22	.....
Weight per 1,000 grains, grams	26.3	36.2	34.6	36.4	25.4	.....	.....	.....	.....	21.2	23.0	22.2	.....
Weight per bushel, pounds	57.7	62.7	61.5	61.5	.....	.....	.....	.....	.....	85	80	.....	.....
Flinty grains.....per cent..	.....	100	100	75	97	.....	.....	.....	.....	.....	.....	.....	.....
On water-free basis:													
Nitrogen.....do.....	2.58	2.59	2.78	2.01	2.03	.....	.....	.....	.....	2.69	2.57	2.34	.....
Protein (N×5.7).....do.....	14.75	14.76	15.84	11.46	11.57	.....	.....	.....	.....	15.33	14.65	13.34	.....
Alcohol-soluble nitrogen, per cent.	1.03	1.23	1.16	.82	.71	.....	.....	.....	.....	1.10	1.05	.92	.....
Gliadin in protein, percent	40	46	41	41	35	.....	.....	.....	.....	41	41	40	.....
Fat.....do.....	1.67	1.82	1.82	1.84	.....	.....	.....	.....	.....	2.16	2.05	2.15	.....
Fiber.....do.....	2.18	2.33	2.43	2.39	.....	.....	.....	.....	.....	2.69	2.62	2.59	.....
Pentosans.....do.....	8.70	8.22	8.49	8.16	8.53	.....	.....	.....	.....	8.37	8.31	8.03	.....
Sugars.....do.....	2.52	3.53	3.21	3.73	3.26	.....	.....	.....	.....	2.89	2.64	2.82	.....
Ash.....do.....	2.05	1.72	1.63	1.63	1.90	.....	.....	.....	.....	2.39	2.30	2.09	.....
Phosphoric acid.....do.....	.96	.79	.68	.70	.89	.....	.....	.....	.....	1.23	1.18	.....	.....
Potash.....do.....	.55	.48	.45	.46	.56	.....	.....	.....	.....	.....	.63	.....	.....
Phosphoric acid in ash, per cent.	46	46	42	43	47	.....	.....	.....	.....	51	51	.....	.....
Potash in ash.....per cent.	25	28	28	29	30	.....	.....	.....	.....	.....	27	.....	.....

## TURKEY WHEAT.

1910 crop.

Determination.	Wheat grown in California on—				Wheat grown in Kansas on—				Wheat grown in Maryland on—			
	California check soil.	California soil.	Kansas soil.	Maryland soil.	California soil.	Kansas soil.	Kansas check soil.	Maryland soil.	California soil.	Kansas soil.	Maryland soil.	Maryland check soil.
Physical properties:												
Water.....per cent..	9.81	9.68	9.67	8.99	9.39	9.03	9.30	9.12	9.00	10.66	9.73	.....
Weight per 1,000 grains, grams	31.2	28.3	34.3	21.5	26.1	22.6	23.3	24.0	28.0	31.5	25.9	.....
Weight per bushel, pounds	60.5	.....	61.8	.....	58.3	56.9	57.2	55.8	.....	57.7	.....	.....
Flinty grains.....per cent..	99	100	70	100	99	100	100	100	0	0	0	.....
On water-free basis:												
Nitrogen.....do.....	2.16	2.39	1.86	2.86	2.80	3.28	3.23	3.12	1.80	1.90	2.05	.....
Protein (N×5.7).....do.....	12.31	13.63	10.60	16.28	15.98	18.73	18.41	17.81	10.27	10.85	11.68	.....
Alcohol-soluble nitrogen, per cent.	.96	1.05	.74	.....	1.23	1.44	1.32	1.29	.....	.75	.....	.....
Gliadin in protein, percent	44	44	40	.....	44	41	41	41	.....	39	.....	.....
Fat.....do.....	2.01	2.13	2.13	2.11	1.86	2.04	1.81	2.02	1.67	1.76	1.78	.....
Fiber.....do.....	2.26	2.15	2.28	2.35	2.72	2.79	2.78	2.80	2.65	3.01	2.63	.....
Pentosans.....do.....	8.27	8.32	8.57	9.25	8.64	8.93	8.78	8.64	8.70	8.54	8.84	.....
Sugars.....do.....	3.40	3.53	3.81	3.43	3.13	3.38	3.11	3.33	2.90	2.99	3.06	.....
Ash.....do.....	1.87	1.84	1.82	2.05	1.99	1.97	2.29	1.97	2.09	2.07	2.22	.....
Phosphoric acid.....do.....	.84	.79	.86	1.02	.85	.81	1.08	.80	.....	1.09	1.21	.....
Potash.....do.....	.60	.61	.55	.65	.61	.66	.69	.64	.....	.57	.61	.....
Phosphoric acid in ash, per cent.	45	43	47	50	43	41	47	41	53	59	.....	.....
Potash in ash.....per cent.	32	33	30	28	31	31	30	30	.....	28	27	.....

<sup>1</sup> Owing to a severe drought the crop failed to mature.

TABLE I.—Composition of wheat grown on different plats of soil in California, in Kansas, and in Maryland in 1909, 1910, 1911, and 1912—Continued.

Determination.	1911 crop. <sup>1</sup>											
	Wheat grown in California on—				Wheat grown in Kansas on—				Wheat grown in Maryland on—			
	California check soil.	California soil.	Kansas soil.	Maryland soil.	California soil.	Kansas soil.	Kansas check soil.	Maryland soil.	California soil.	Kansas soil.	Maryland soil.	Maryland check soil.
Physical properties:												
Water.....per cent.					9.00	9.30	8.28	8.79	8.83	8.07	8.93	8.73
Weight per 1,000 grains....grams.	38.4	34.6	37.7	23.5	12.9	13.3	12.5	13.8	27.4	29.4	27.1	26.4
Weight per bushel.....pounds.									60.5	62.2		59.9
Flinty grains.....per cent.	89	51	46	100	100	98	97	98	25	20		50
On water-free basis:												
Nitrogen.....do.					3.70	4.09	4.07	3.97	2.00	2.20	2.37	2.31
Protein (N×5.7).....do.	12.04	10.56	9.61	13.20	21.11	23.31	23.18	22.62	11.38	12.52	13.52	13.18
Alcohol-soluble nitrogen.....do.	.93	.76	.70	.80					.85	.88	1.04	.96
Gliadin in protein.....do.	44	43	42	35					43	40	44	41
Fat.....do.					1.94	1.95	1.83	2.12	2.04	2.05	1.83	1.87
Fiber.....do.					2.95	2.94	3.17	3.21	2.41	2.33	2.49	2.44
Pentosans.....do.					8.84	9.12	9.57	9.12	8.08	8.22	8.25	8.39
Sugars.....do.									3.25	3.45	3.33	3.34
Ash.....do.	1.60	1.88	1.76	1.78	2.58	2.56	2.78	2.09	2.23	2.20	2.10	2.17
Phosphoric acid.....do.					1.14	1.18	.86	1.24	1.16	1.09	1.17	
Potash.....do.					.69			.64	.67	.65	.65	.67
Phosphoric acid in ash.....do.					44	46		41	56	53	52	54
Potash in ash.....do.					27			30	30	30	31	31
1912 crop.												
Physical properties:												
Water.....per cent.	8.43	8.29	8.67	8.70	10.55	10.18	10.30	10.45	10.22	9.65	10.13	10.17
Weight per 1,000 grains....grams.	29.3	30.5	31.8	23.9	22.0	21.4	28.6	16.2	25.7	25.7	19.19	22.4
Weight per bushel.....pounds.		64.3	65.1			57.7			60.1	60.3		
Flinty grains.....per cent.	90	98	98	99	98				30	75		70
On water-free basis:												
Nitrogen.....do.	2.05	2.24	2.40	3.17	2.68	2.78	3.62	3.29	1.85	2.12	2.28	2.05
Protein (N×5.7).....do.	11.68	12.77	13.68	18.07	15.29	15.87	20.65	18.78	10.54	12.11	13	11.68
Alcohol-soluble nitrogen.....do.	.85	.97	1.02	1.40	1.14	1.20	1.64	1.35	.70	.85	.89	.83
Gliadin in protein.....do.	41	43	44	42	43	45	41	38	40	39	40	
Fat.....do.	1.89	1.93	1.88	2.05	1.88	2.17	1.82	2.01	1.89	1.97	1.88	2
Fiber.....do.	2.28	2.20	2.28	2.69	2.68	2.65	2.48	2.24	2.72	2.53	2.88	2.87
Pentosans.....do.	8.08	7.95	8.05	8.70	8.31	8.27	8.52	8.98	8.44	8.62	9.30	9.03
Sugars.....do.	3.50	3.78	3.80	3.91	3.37	3.41	3.12		2.95	3.08		3.32
Ash.....do.	2.07	2.14	2.18	2.20	2.47	2.20	2.45	2.83	2.24	2.24	2.48	2.46
Phosphoric acid.....do.	1.02	1.07	1.09	1.03	1.23	1.02	1.26	1.31	1.14	1.19	1.27	1.24
Potash.....do.	.62	.62	.62	.59	.74	.67	.66	.79	.71	.72	.80	.79
Phosphoric acid in ash.....do.	52	50	50	47	50	46	51	46	51	53	51	50
Potash in ash.....do.	30	30	29	27	30	30	27	28	32	32	32	32

<sup>1</sup> The data for the 1911 samples grown in California were furnished by Prof. Shaw, of the University of California, under whose supervision the field work in that State was conducted.

TABLE II.—Composition of wheat grown on plats of California, Kansas, and Maryland soils in California, in Kansas, and in Maryland.

Determination.	Analysis of wheat grown on—								
	California soil in—			Kansas soil in—			Maryland soil in—		
	California.	Kansas.	Maryland.	California.	Kansas.	Maryland.	California.	Kansas.	Maryland.
1909.									
Physical properties:									
Water.....per cent.	8.98		9.56	9.00		9.48	8.88		9.22
Weight per 1,000 grains.....grams.	34.6		21.2	36.4		23.0	25.4		22.2
Weight per bushel.....pounds.	61.5			61.5					
Flinty grains.....per cent.	100		85	75		80	97		
On water-free basis:									
Nitrogen.....do.	2.78		2.69	2.01		2.57	2.03		2.34
Protein (N X 5.7).....do.	15.84		15.33	11.46		14.65	11.57		13.34
Alcohol-soluble nitrogen.....do.	1.16		1.10	.82		1.05	.71		.92
Gladiin in protein.....do.	41		41	41		41	35		40
Fat.....do.	1.82		2.16	1.82		2.05	1.84		2.15
Fiber.....do.	2.33		2.69	2.43		2.62	2.39		2.59
Pentosans.....do.	8.49		8.37	8.10		8.31	8.53		9.03
Sugars.....do.	3.21		2.89	3.73		2.04	3.26		2.82
Ash.....do.	1.63		2.39	1.03		2.30	1.90		2.09
Phosphoric acid.....do.	.68		1.23	.70		1.18	.89		
Potash.....do.	.45			.46		.63	.56		
Phosphoric acid in ash.....do.	42		51	43		51	47		
Potash in ash.....do.	28			29		27	30		
1910.									
Physical properties:									
Water.....per cent.	9.00	9.39	9.00	9.67	9.03	10.66	8.99	9.12	9.73
Weight per 1,000 grains.....grams.	28.3	26.1	28.0	34.3	22.6	31.5	21.5	24.0	25.9
Weight per bushel.....pounds.	58.3			61.8	56.9	57.7		55.8	
Flinty grains.....per cent.	100	99	0	70	100	0	100	100	0
On water-free basis:									
Nitrogen.....do.	2.39	2.80	1.80	1.86	3.28	1.90	2.86	3.12	2.05
Protein (N X 5.7).....do.	13.63	15.98	10.27	10.60	18.73	10.85	16.28	17.81	11.63
Alcohol-soluble nitrogen.....do.	1.05	1.23		.74	1.44	.75		1.29	
Gladiin in protein.....do.	44	44		40	41	39		41	
Fat.....do.	2.13	1.86	1.67	2.13	2.04	1.76	2.11	2.02	1.78
Fiber.....do.	2.15	2.72	2.65	2.28	2.79	3.01	2.35	2.80	2.65
Pentosans.....do.	8.32	8.64	8.70	8.57	8.93	8.54	9.25	8.64	8.84
Sugars.....do.	3.53	3.13	2.90	3.81	3.38	2.99	3.43	3.33	3.06
Ash.....do.	1.84	1.99	2.09	1.82	1.97	2.07	2.05	1.97	2.22
Phosphoric acid.....do.	.79	.85		.86	.81	1.09	1.02	.80	1.21
Potash.....do.	.61	.61		.55	.66	.57	.65	.64	.61
Phosphoric acid in ash.....do.	43	43		47	41	53	50	41	59
Potash in ash.....do.	33	31		30	31	28	28	30	27
1911.									
Physical properties:									
Water.....per cent.		9.00	8.83		9.30	8.97		8.79	8.93
Weight per 1,000 grains.....grams.	34.6	12.9	27.4	37.7	13.3	29.4	23.5	13.8	27.1
Weight per bushel.....pounds.			60.5			62.2			
Flinty grains.....per cent.	51	100	25	46	98	20	100	98	
On water-free basis:									
Nitrogen.....do.		3.70	2.00		4.09	2.20		3.97	2.37
Protein (N X 5.7).....do.		10.56	21.11	11.38	9.61	12.52	13.20	22.62	13.52
Alcohol-soluble nitrogen.....do.		.76	.85	.70		.88	.80		1.04
Gladiin in protein.....do.		43	43	42		40	35		44
Fat.....do.		1.94	2.04		1.95	2.05		2.12	1.83
Fiber.....do.		2.95	2.41		2.94	2.33		3.21	2.49
Pentosans.....do.		8.84	8.08		9.12	8.22		9.12	8.25
Sugars.....do.			3.25			3.45			3.33
Ash.....do.		1.88	2.23	1.76	2.56	2.20	1.78	2.09	2.10
Phosphoric acid.....do.		1.14	1.24		1.18	1.16		.86	1.09
Potash.....do.		.69	.67			.65		.64	.65
Phosphoric acid in ash.....do.		44	56		46	53		41	52
Potash in ash.....do.		27	30			30		30	31

TABLE II.—Composition of wheat grown on plats of California, Kansas, and Maryland soils in California, in Kansas, and in Maryland—Continued.

1912.

Determination.	Analysis of wheat grown on—								
	California soil in—			Kansas soil in—			Maryland soil in—		
	California.	Kansas.	Maryland.	California.	Kansas.	Maryland.	California.	Kansas.	Maryland.
Physical properties:									
Water.....per cent..	8.29	10.55	10.22	8.67	10.18	9.65	8.70	10.45	10.13
Weight per 1,000 grains.....grams..	30.5	22.0	25.7	31.8	21.4	25.7	23.9	16.2	19.9
Weight per bushel.....pounds..	64.3	.....	60.1	65.1	57.7	60.3	.....	.....	.....
Flinty grains.....per cent..	98	98	30	98	100	75	99	.....	.....
On water-free basis:									
Nitrogen.....do.....	2.24	2.68	1.85	2.40	2.78	2.12	3.17	3.29	2.28
Protein (N X 5.7).....do.....	12.77	15.29	10.54	13.68	15.87	12.11	18.07	18.78	13
Alcohol-soluble nitrogen.....do.....	.97	1.14	.70	1.02	1.20	.85	1.40	1.35	89
Gliadin in protein.....do.....	43	42	38	43	43	40	44	41	39
Fat.....do.....	1.93	1.88	1.89	1.88	2.17	1.97	2.05	2.01	1.88
Fiber.....do.....	2.20	2.68	2.72	2.28	2.65	2.53	2.69	3.24	2.88
Pentosans.....do.....	7.95	8.31	8.44	8.05	8.27	8.62	8.70	8.98	9.36
Sugars.....do.....	3.78	3.37	2.95	3.80	3.41	3.08	3.91	.....	.....
Ash.....do.....	2.14	2.47	2.24	2.18	2.20	2.24	2.20	2.83	2.48
Phosphoric acid.....do.....	1.07	1.23	1.14	1.09	1.02	1.19	1.03	1.31	1.27
Potash.....do.....	.63	.74	.71	.62	.67	.72	.59	.79	.80
Phosphoric acid in ash.....do.....	50	50	51	50	46	53	47	46	51
Potash in ash.....do.....	30	30	32	29	30	32	27	28	32

TABLE III.—Averages and extremes in wheat grown on plats of California, Kansas, and Maryland soils in California, in Kansas, and in Maryland.<sup>1</sup>

Determination.	California.				Kansas.				Maryland.			
	Averages.		Extremes.		Averages.		Extremes.		Averages.		Extremes.	
	Mean.	Divergence from mean.	Minimum.	Maximum.	Mean.	Divergence from mean.	Minimum.	Maximum.	Mean.	Divergence from mean.	Minimum.	Maximum.
Physical properties:												
Water.....per cent..	8.98	0.31	8.29	9.68	9.53	0.57	8.79	10.55	9.53	0.46	8.83	10.66
Weight per 1,000 grains, grams.....	30.2	4.5	21.5	37.7	19.1	4.5	12.9	26.1	25.6	2.7	19.9	31.5
Weight per bushel, pounds.....	62.8	1.5	61.5	65.1	57.2	.8	55.8	58.3	60.1	1	57.7	62.2
Flinty grains, per cent..	86	17	46	100	99	1	98	100	35	30	0	85
On water-free basis:												
Nitrogen.....per cent..	2.42	.35	1.86	3.17	3.30	.41	2.68	4.09	2.18	.23	1.80	2.69
Protein (N X 5.7), per cent.	13.11	2.01	9.61	18.07	18.83	2.34	15.29	23.31	12.43	1.29	10.27	15.33
Alcohol-soluble nitrogen.....per cent..	.92	.18	.70	1.40	1.27	.09	1.14	1.44	.90	.10	.70	1.10
Gliadin in protein, per cent.....	41	2	35	44	42	1	41	44	40	1	38	44
Fat.....per cent..	1.97	.12	1.82	2.13	2	.08	1.86	2.17	1.94	.14	1.67	2.16
Fiber.....do.....	2.34	.11	2.15	2.69	2.89	.18	2.65	3.21	2.63	.13	2.33	3.01
Pentosans.....do.....	8.45	.22	7.95	9.25	8.76	.26	8.27	9.12	8.56	.29	8.08	9.36
Sugars.....do.....	3.61	.22	3.21	3.91	3.32	.08	3.13	3.41	3.03	.18	2.64	3.45
Ash.....do.....	1.90	.16	1.63	2.20	2.30	.28	1.97	2.83	2.22	.09	2.07	2.48
Phosphoric acid.....do.....	.90	.13	.68	1.09	1.02	.17	.80	1.31	1.18	.05	1.09	1.27
Potash.....do.....	.57	.06	.45	.65	.68	.04	.61	.79	.67	.05	.57	.80
Phosphoric acid in ash, per cent.....	47	2	42	50	45	4	41	50	53	2	51	59
Potash in ash, per cent..	29	1	27	33	30	1	27	31	30	2	27	32

<sup>1</sup> Not including check plats.

TABLE IV.—Averages and extremes in wheat grown in California, in Kansas, and in Maryland on plats of California, Kansas, and Maryland soils.<sup>1</sup>

Determination.	California soil.				Kansas soil.				Maryland soil.			
	Averages.		Extremes.		Averages.		Extremes.		Averages.		Extremes.	
	Mean.	Divergence from mean.	Minimum.	Maximum.	Mean.	Divergence from mean.	Minimum.	Maximum.	Mean.	Divergence from mean.	Minimum.	Maximum.
Physical properties:												
Water.....per cent..	9.35	0.53	8.29	10.22	9.46	0.47	8.67	10.66	9.29	0.48	8.70	10.45
Weight per 1,000 grains, grams.....	26.5	4.5	12.9	34.6	27.9	6.1	13.3	37.7	22.1	3.1	13.8	27.1
Weight per bushel, pounds.....	60.9	1.5	58.3	64.3	60.4	2.2	56.9	65.1	(2)	(2)	(0)	(2)
Flinty grains, per cent..	71	33	0	100	69	26	0	100	85	24	0	100
On water-free basis:												
Nitrogen.....per cent..	2.48	.44	1.80	3.70	2.52	.53	1.86	4.09	2.75	.53	2.03	3.97
Protein (N X 5.7), per cent..	13.88	2.56	10.27	21.11	13.94	3.05	9.61	23.31	15.44	2.97	11.57	22.62
Alcohol-soluble nitrogen.....per cent..	1	.16	.70	1.23	.94	.18	.70	1.44	1.05	.22	.71	1.40
Gladi in protein, per cent.....	42	1	38	44	41	1	39	43	40	3	35	44
Fat.....per cent..	1.93	.11	1.67	2.16	1.98	.11	1.76	2.17	1.97	.12	1.78	2.15
Fiber.....do.....	2.55	.22	2.15	2.95	2.59	.22	2.28	3.01	2.73	.24	2.35	3.24
Pentosans.....do.....	8.41	.21	7.95	8.84	8.48	.28	8.05	9.12	8.87	.28	8.25	9.36
Sugars.....do.....	3.33	.28	2.89	3.90	3.48	.24	2.99	3.81	3.30	.22	2.82	3.91
Ash.....do.....	2.13	.23	1.63	2.58	2.08	.11	1.63	2.56	2.16	.20	1.78	2.83
Phosphoric acid.....do.....	1.04	.18	.68	1.24	1.03	.15	.70	1.19	1.05	.15	.80	1.31
Potash.....do.....	.64	.06	.45	.74	.61	.07	.46	.72	.66	.06	.56	.80
Phosphoric acid in ash, per cent.....	48	4	42	56	48	4	41	53	48	4	41	59
Potash in ash, per cent..	30	1	27	33	29	1	27	32	29	2	27	32

<sup>1</sup> Not including check plats.<sup>2</sup> Only 1 sample.TABLE V.—Averages and extremes for the years 1909, 1910, 1911, and 1912 in wheat on disturbed and undisturbed plats<sup>1</sup> for all localities (California, Kansas, and Maryland) and years.

* Determination.	Disturbed.				Undisturbed.			
	Averages.		Extremes.		Averages.		Extremes.	
	Mean.	Divergence from mean.	Minimum.	Maximum.	Mean.	Divergence from mean.	Minimum.	Maximum.
Physical properties:								
Water.....per cent..	9.31	0.51	8.29	10.18	9.33	0.65	8.28	10.30
Weight per 1,000 grains, grams.....	25.8	4.9	13.3	34.6	27.6	5.7	12.5	38.4
Weight per bushel, pounds.....	60.9	1.5	58.3	64.3	60.4	2.2	56.9	65.1
Flinty grains.....per cent..	92	12	51	100	96	4	89	100
On water-free basis:								
Nitrogen.....per cent..	2.78	.45	2.24	4.09	2.76	.66	2.05	4.07
Protein (N X 5.7).....do.....	15.25	2.84	10.56	23.31	15.32	3.62	11.68	23.18
Alcoholic-soluble nitrogen, per cent.....	1.06	.15	.76	1.44	1.09	.23	.83	1.64
Gladi in protein.....per cent..	42	1	39	44	43	2	40	46
Fat.....do.....	1.97	.11	1.82	2.17	1.86	.08	1.67	2.01
Fiber.....do.....	2.55	.26	2.15	2.94	2.56	.29	2.18	3.17
Pentosans.....do.....	8.59	.41	7.95	9.36	8.61	.35	8.08	9.57
Sugars.....do.....	3.44	.14	3.21	3.78	3.34	.14	3.11	3.56
Ash.....do.....	2.09	.23	1.63	2.56	2.16	.30	1.60	2.78
Phosphoric acid.....do.....	.96	.17	.68	1.27	1.06	.15	.79	1.26
Potash.....do.....	.64	.06	.45	.80	.64	.07	.48	.79
Phosphoric acid in ash.....do.....	46	4	41	52	49	3	45	54
Potash in ash.....do.....	31	1	28	33	30	1	27	32

<sup>1</sup> Only data that are strictly comparable are used. Disturbed-plat data are used only if the determinations for the corresponding check plats were also made, and vice versa.

## PHYSICAL CHARACTERISTICS

## WEIGHT OF 1,000 GRAINS OF WHEAT

In California the grains were almost uniformly plump and heavy, not varying far from 30 grams for each thousand, except in the case of the samples grown on the soil obtained from Maryland. In Kansas they were less plump, 1,000 grains weighing about 23 grams in 1910, 13 grams in 1911, and 20 grams in 1912. In Maryland the weight of 1,000 grains was quite uniform throughout the series of four years. As a rule, the size of the grains in each locality for each year was uniform, irrespective of the type of soil in which they grew. There were, however, a few notable exceptions to this rule: The grain grown on Maryland soil in each year from 1909 to 1912 in California, as well as that grown on the Maryland soil in 1912 in Kansas, was decidedly lighter in weight than that grown in the same locality on the other soils. This would seem to indicate that some soils play an important part in influencing the size of the grain.

Between the localities there was usually a much greater difference in the weight of 1,000 grains than was noted between the soils. (See Table II.) The weight of 1,000 grains, then, is distinctly dependent, as a rule, on climatic or seasonal conditions rather than on soil characteristics. The fact that environment plays the chief rôle in influencing the weight is again brought out in the tables of averages, which show a great difference, for example, 30.2, 19.1, and 25.6 grams for California, Kansas, and Maryland, respectively, when averaged by localities (see Table III), and a relative uniformity of 26.5, 27.9, and 22.1 grams, respectively, when averaged by source of soil (see Table IV).

Table I shows that in about 80 per cent of the samples investigated the weight of 1,000 grains of seed grown on different soils in any one locality was sufficiently uniform to permit the conclusion that climate and not soil is the chief factor affecting the size of the grain. From Table III it is seen that the California-grown samples averaged the heaviest and the Kansas-grown samples the lightest.

## WEIGHT OF ONE BUSHEL OF WHEAT

The weight of a bushel of wheat runs more or less parallel with the weight of 1,000 grains. If the samples weighing over 61 pounds to the bushel are compared with those weighing less than 60 pounds, it will be found that the weight of 1,000 grains of the former was, on an average, 33.4 grams, and that of the latter, 25 grams. In many cases, owing to the small amount of material, it was impossible to make a weight-by-bushel determination.

## FLINTY GRAINS

Classifying the grains of each sample into those which were wholly dark or flinty and those which appeared to be light brown or mealy, a remarkable uniformity is found in the groups arranged by locality in

which they grew (see Table I) and a dissimilarity in groups arranged by the source of soil (see Table II). The averages by localities (see Table III) differ greatly, being 86, 99, and 35 per cent for California, Kansas, and Maryland, respectively. The averages by soils are very uniform, being 71, 69, and 85 per cent, respectively. (See Table IV.)

These averages do not show the great variations actually found in the different regions in any one year, for seasonal variations of the individual localities tend to equalize the averages. In Table I, for example, while the samples grown in California and Kansas in 1910 in each of the three soils were for the most part flinty, those grown in the three soils in Maryland were all more or less starchy or mealy. Similar figures are noted in 1911, when the Kansas samples grown on all three soils yielded wheat which was practically 100 per cent flinty, while on the same soils in Maryland the percentage of flinty kernels was less than half as great.

#### CHEMICAL CONSTITUENTS

In considering the composition of the wheat it will be seen that many of the organic and inorganic constituents undergo as great variations as have already been noted with respect to the physical characteristics. On the other hand, there are a number of these constituents which showed very little variation, or no regularity in such variations as exist. Among those showing but little variation may be mentioned the gliadin number and the potash in the ash, and among those showing no pronounced regularity in the variations are the fat, fiber, pentosans, and sugars. With those exhibiting variations of a regular character belong particularly the nitrogen and protein, the ash, the phosphoric acid, and the phosphoric acid in the ash.

#### PROTEIN

As the protein of wheat is its most important constituent, it will be of more than usual interest to note the changes produced by difference of soil and by change of environment. As a rule, there was a remarkable uniformity each year among the samples grown in any one locality, independent of the soil upon which they grew. Thus, in 1910, 1911, and 1912 the protein in wheat grown in California was almost uniformly low, about 13 per cent; in Maryland it was also low, about 11 per cent; while in Kansas it was high, nearly 18 per cent. This fact is more clearly brought out in Table III, which shows the average protein content to be 13.11, 18.83, and 12.43 per cent for California, Kansas, and Maryland, respectively.

In Table IV, where the results are arranged according to source of soil, it will be seen that the wheats grown on California soil in all three localities had an average protein content of 13.88 per cent, those grown on Kansas soil, 13.94 per cent, and those on Maryland soil, 15.44 per cent. This shows a rather striking uniformity and again emphasizes the rela-

tively small rôle played by the soil in influencing the protein content of wheat. There was a greater similarity between the protein contents of the samples grown in Maryland and California, both relatively humid regions, than between the protein contents of samples from either of these localities and those from Kansas, which has a comparatively dry climate.

There are a few exceptions, however, to the rule that soil influences the composition of wheat to only a slight degree. Among the most striking of these were the protein results obtained in 1909 in California on California soil, in 1910 and 1912 in California on Maryland soil, as well as in Kansas on Kansas check soil; that is, 4 out of 42 cases did not follow the general rule. Since about 90 per cent of the results obtained followed the general rule, and the exceptions noted were in different localities and on different soils and not always on the same soil in any locality, it is probably safe to assume that the contrary results given by the other 10 per cent of samples were accidental. These few exceptions among the prevailing regularities may serve to emphasize the fact, too frequently overlooked in plat experiments of this kind where many factors may affect the results, that a regularity needs to be traced through a great number of individual instances before it is safe to draw conclusions from it. Thus, in this experiment a consideration of the data from the 1909 crop alone might show that the soil has a marked determining influence upon the protein content and that the California soil tends to produce a wheat of relatively high protein content. That such a conclusion would be erroneous is evidenced by practically all the data of the three following years, for in no other case during 1910, 1911, and 1912 was there a larger amount of protein in wheat grown on the California soil than in that grown on the two other soils. In fact, those wheats were invariably lower in protein content.

While these exceptions may be considered as purely accidental, the following question is suggested by such variations from the rule: Is there in the physical, chemical, or biological characteristics of the soil a real difference which at first exerts a determining influence on the composition of the crop, but which may be obliterated in the course of a year or two after putting the soil down in a different locality? Some weight is lent to such a hypothesis by the fact that the slight differences in protein content in the crops grown in Maryland the first year after the exchange of soils were much the same as the exceptionally great differences in the crops grown in California. Unfortunately, the Kansas crop was a complete failure, and it is impossible, therefore, to know in what way the soil there would have influenced the composition of the crop during the first year. To answer this question, more observations during the first few years of similar soil exchange experiments would be necessary, using larger plats to partly eliminate any tendency for soils to equalize after being together in one locality, if such a tendency does exist.



It seems justifiable to conclude that climate is the principal factor influencing the protein content of wheat, and that soils, when used as in this experiment, have little or no influence.

#### GLIADIN IN PROTEIN

With very few exceptions, the amount of alcohol-soluble nitrogen or gliadin bore a close relation to that of total nitrogen. The percentage of gliadin in the wheat grown on the different soils in the three localities during the years 1909 to 1912 remained practically constant at 41 per cent, except in the case of wheat grown on Maryland soil and on California check soil in California in 1909, and on Maryland soil in California in 1911. These 3 exceptions out of 42 samples can not be explained and must be assumed to be accidental. From Table II it would seem that those samples grown on Maryland soil in California in 1909, 1911, and 1912 and in Maryland in 1912 formed exceptions to the rule. When general averages are considered, however, practically no differences in gliadin number due either to difference of soils or to change of seasonal conditions are noted. Table III gives the average gliadin numbers of the samples grown on each of the three soils in California as 41; in Kansas, 42; and in Maryland, 40. Table IV shows the gliadin number of the wheats grown on California soil in each of the three localities to be 42; on Kansas soil, 41; and on Maryland soil, 40. There seems to be a slight tendency for the Maryland soil to be low in gliadin. The differences are, however, small and probably no weight should be given them.

#### FAT

The amounts of fat agreed very closely in the case of wheat grown on the different soils in any one locality, only 3 out of 42 samples showing a greater variation than 0.2 per cent, which may be assumed to be the limit of error for fat determinations. When averaged by locality, the results were 1.97, 2.00, and 1.94 per cent for wheat grown in California, Kansas, and Maryland, respectively. When averaged by source of soils, the results were 1.93, 1.98, and 1.97 per cent for samples grown on California, Kansas, and Maryland soils, respectively. The results taken as a whole indicate that fat is not affected to any great extent by climatic or soil conditions.

#### FIBER

The fiber showed a somewhat greater variation in amount than did the fat. The results as a whole indicate that a greater influence is exerted by seasonal or climatic changes than by differences in soils. This is shown in Table III, with the average fiber content of 2.34, 2.89, and 2.63 per cent in the wheats grown on the three soils in California, Kansas, and Maryland, respectively.

The wheat grown in the three localities on California soil gave 2.55 per cent of fiber, on Kansas soil, 2.59, and on Maryland soil, 2.73. (See

Table IV.) These averages agree with one another more closely than do those in Table III, proving that soils play a minor rôle in influencing the fiber content.

#### PENTOSANS

The pentosan content followed generally the fiber content, being high where the fiber content was high and low where the fiber content was low.

#### SUGARS

The sugar content of the samples grown in California was somewhat higher than that of those grown in Kansas or in Maryland.

#### ASH

If soil itself has any influence on the composition of the wheat, it is reasonable to expect that the mineral constituents especially will be thus influenced. Even here, however, in the case of ash, the soil factor is a minor or negligible one. There was a decided regularity in the ash content, and, like the physical properties and the protein content, this regularity consisted in an approximately uniform ash content of the samples grown during any one year in any one locality. Thus, during each of the four years California produced from all soils crops with a low ash content of about 1.9 per cent, while Kansas produced crops relatively higher in ash, averaging 2.30 per cent, and Maryland nearly as high, varying somewhat, however, from year to year, with an average of 2.22 per cent. The average ash content of all crops grown on each of the three soils, irrespective of the locality, showed but slight variation, being 2.13, 2.08, and 2.16 per cent for California, Kansas, and Maryland soils, respectively.

#### PHOSPHORIC-ACID CONTENT OF THE WHOLE WHEAT AND OF THE ASH

In most cases the amount of phosphoric acid rose or fell in the same proportion as the ash, so that the percentage of phosphoric acid in the ash remained practically constant, averaging 47 per cent for California, 45 per cent for Kansas, and varying from 41 to 51 per cent in these two localities. The crops grown in Maryland, however, on all soils had a strangely high amount of phosphoric acid, averaging 53 per cent of the ash and varying from 51 to 59 per cent. There is no explanation for the fact that in Maryland all the soils used in this experiment supplied to the grain mineral constituents with a percentage of phosphoric acid much higher than that supplied by the same soils in California and in Kansas. It was apparently due to some climatic or seasonal conditions prevailing in Maryland. The kind of soil did not, however, affect the amount of phosphoric acid in the wheat or in the ash, for Table IV shows that the average in the wheat grown in the three localities on plats of California soil was 1.04 per cent, on plats of Kansas soil, 1.03 per cent, and on plats of Maryland soil, 1.05 per cent, and the phosphoric acid in the ash was 48 per cent in each case.

## POTASH CONTENT OF THE WHOLE WHEAT AND OF THE ASH

The potash in the wheat, like the total ash, was seemingly influenced more by climatic and seasonal variations than by the soil, so that the amount of potash in all samples rose or fell in practically the same proportion as the amount of total ash, and the percentage of potash in the ash—about 30 per cent—remained very nearly constant for all localities, soils, and seasons included in the experiment. This is further shown by the similarity of the averages, whether by locality (see Table III), with averages of 29, 30, and 30 per cent for California, Kansas, and Maryland, respectively, or by soils (see Table IV) with averages of 30, 29, and 29 per cent, respectively.

## CORRELATION BETWEEN PHYSICAL PROPERTIES AND CHEMICAL CONSTITUENTS

Although the relationship or interdependence between the physical properties and chemical constituents does not show in these results as markedly as might be expected, such relationships may be distinctly traced in some of the constituents. Thus, as has often been pointed out by others, a distinct correlation exists between the protein content and the physical appearance or between the protein content and the weight of 1,000 grains, high protein being more or less parallel with flintiness and with lightness of grains. The table of averages (see Table III) shows that the Kansas samples, containing 18.83 per cent of protein, averaged 99 per cent of flinty grains and weighed at the rate of 19.1 grams for 1,000 grains, while the Maryland samples, containing 12.43 per cent of protein, averaged but 35 per cent of flinty kernels and weighed 25.6 grams for 1,000 grains, and the California samples, containing 13.11 per cent of protein, averaged 86 per cent of flinty grains and weighed as high as 30.2 grams for 1,000 grains. The results in Table IV show a similar tendency in these respects, the samples grown on Maryland soils in the three localities being somewhat richer in protein and having at the same time more flinty kernels and weighing less for each 1,000 grains than the samples grown on California or Kansas soils. The differences in this case, however, were very much less notable than those due to climatic variations. (See Table III.) There was a less noticeable parallelism between the fiber and pentosans, a high fiber content, as a rule, being accompanied by a high pentosan content, and vice versa. The California-grown samples, which were the heaviest, contained the smallest amount of fiber and pentosans, while the Kansas samples, which were the lightest, contained the greatest amount.

The fact that the ash and protein contents were low in the California-grown samples and high in the Kansas-grown samples might lead one to expect that the ash was a function of the protein content. This is not borne out by an examination of Table III, where it is seen that the ash

of the samples grown in Maryland was appreciably higher than that of the samples grown in California, while the protein of the former was less than that of the latter. On the other hand, the ash content of the Kansas samples was only slightly higher than that of the Maryland-grown samples, although the protein content of the former was 50 per cent higher than that of the latter.

#### COMPARISON BETWEEN RESULTS FROM DISTURBED AND UNDISTURBED PLATS OF THE SAME SOIL

Attention has thus far been directed primarily to the composition of the wheat samples grown for several years in each locality on each of the three soil plats which had been taken up in 3-inch layers and interchanged among the three localities. As previously mentioned, a check plat of equal size, in which the soil had not been disturbed, was planted each year in each locality, and samples from it were analyzed for comparison. A fear that manipulation of the soil would produce abnormal conditions, influencing the character of the crop, was not justified by these results (Table V), at least not as evidenced by the physical appearance and the chemical composition. The slight differences between the crops from the disturbed and undisturbed plats of the same soil are apparently either accidental or due to errors in sampling or in analysis. This is further borne out by the results from both the seed-exchange experiments<sup>1</sup> and from the soil-exchange experiments (pp. 278-281). It is simply a verification of the conclusion already drawn, that the soil factor plays but a very subordinate part or is entirely devoid of influence in determining these characteristics in the crop.

Such great differences exist in respect to one constituent, however, that they must be classed as exceptions to the rule. The percentage of phosphoric acid averaged 0.96 per cent in the samples from disturbed plats and 1.06 per cent in those from undisturbed plats, or, if expressed as the percentage of phosphoric acid in the ash, it is 46 and 49 per cent, respectively. It might seem that the undisturbed soil could give a little more phosphoric acid to the grain than the disturbed soil. These differences, being only slightly greater than the limit of error in analytical work, probably have no significance.

#### CONCLUSIONS

As is to be expected in plat work in the field, especially with such small plats as were used for these experiments, there are many variations in the results which seem accidental, in that they can not be interpreted according to any definite law. There are, however, certain variations which appear with such regularity that important conclusions may be drawn from them.

An inspection of the tables should show whether climatic conditions or soil characteristics have a strong determining influence upon the

---

<sup>1</sup> Le Clerc and Leavitt. *Op. cit.*

properties or composition of the crop. If the adjacent data in Table I under each locality are similar and distinctly unlike the corresponding group in another region, it is evident that the locality—that is, the climate—has exerted a strong influence. Likewise, if a similarity exists in the data in the adjacent columns in Table II as regards crops from the same soil and there is a distinct difference between them and the corresponding data from other soils, it is clear that the soils in themselves have a determining influence, regardless of the locality in which the soils happen to be.

To avoid erroneous conclusions concerning any property or constituent, due to accidental differences occurring in individual groups of data, it is necessary to make a survey of all the data on hand regarding that property or constituent. In a measure the averages drawn from the several groups of data furnish quantitative values which may indicate the persistence or the nonpersistence of such differences. The average divergences from these means, together with the minima and the maxima, supply further quantitative evidence along this line. Such averages and the corresponding minima and maxima are brought together in Tables III, IV, and V.

This experiment, covering a period of four years, in which three fairly good wheat soils, one each from California, Kansas, and Maryland, were put down side by side in each of these three localities and cropped with the same variety of wheat, shows that the soil does not exert the chief or preponderating influence in determining the physical properties or the chemical constituents of the grain crop. No attempt has been made to trace out from these experiments the manner in which the climatic factors thus exert the chief determining influence on the composition of the wheat crop. The following possibilities may, however, be considered:

(1) Differences in humidity may cause a difference in the transpiration of the plants, which in turn may react on the composition of the crop.

(2) Variations in the amount and distribution of sunlight may influence diversely the photosynthesis of the plants.

(3) Differences in temperature and in the succession of hot and cold periods may cause varying vegetative activities in the plants.

(4) The climatic differences, such as the humidity, rainfall, temperature, and sunlight, may bring about changes in the physical, chemical, or biological characteristics of the soil which in turn may react on the crop.

From this it should not be assumed that it is impossible for soil which has been transferred from one locality to another to become so changed by climatic environment that the character of the wheat grown thereon would be approximately the same as that grown in soil belonging to the second locality. This has been suggested to explain the facts observed during this experiment—namely, that wheats grown on the three soils in Kansas are very different from the same variety of wheat grown

on the same soils transported to Maryland. In view of the further fact, generally accepted by agriculturists, that the same variety of wheat grown over certain large areas having similar climatic conditions possesses approximately the same physical and chemical characteristics, notwithstanding the inherent differences in soil on which they were grown or the differences of fertilizers applied to these soils, it would seem that climate plays a greater rôle than soils as such in influencing the composition of wheat.

Of the biological factors, those bearing on nitrification might be the most influential in affecting the protein content of the crop. Yet it is a noteworthy fact that the application of nitrate as a fertilizer increases the protein content of the crop to only a slight degree. Considering the great difference existing between the protein of the Maryland and Kansas crops, it may therefore be concluded that even if nitrification were greater in Maryland soil transferred to Kansas than in Maryland soil in Maryland, that fact would not be sufficient to explain the wide variation between the composition of the wheat grown on the four plats in Maryland and on the four plats in Kansas.

It is also shown that the crops from the plats which had been taken up in 3-inch layers and replaced had approximately the same physical and chemical characteristics throughout as the crops from the corresponding plats which had not been thus disturbed. On the other hand, it is shown that the climatic factors collectively have a strong determining influence, especially upon the crude-protein content, the ash content, and the percentage of phosphoric acid in the ash. The results from this experiment thus harmonize with the findings previously published<sup>1</sup>—namely, that environment rather than what has been usually termed heredity is the major factor in determining the physical and chemical characteristics of the wheat crop. They indicate, further, that it is the climatic environment which exercises the primary influence of the environmental factors.

---

<sup>1</sup> Le Clerc and Leavitt. *Op. cit.*



# A DROUGHT-RESISTING ADAPTATION IN SEEDLINGS OF HOPI MAIZE

By G. N. COLLINS,

*Botanist, Office of Acclimatization and Adaptation of Crop Plants,  
Bureau of Plant Industry*

## INTRODUCTION

A study of the maize grown by the Hopi, Zuni, and Navajo Indians of New Mexico and Arizona has brought to light an adaptive character that promises to be of economic importance in dry regions where germination is uncertain.

These southwestern Indians have preserved from pre-Columbian times a type of maize able to produce fair crops in regions where the better known varieties of the East fail for lack of sufficient water. An important factor in the drought resistance of this type of corn is its ability to force the growing shoot of the seedling to the surface of the soil when planted at a depth of a foot or more. At such depths less specialized varieties die before reaching the surface.

The literature of corn contains reports of many experiments conducted to determine the proper depth of planting, but the results are confusing and contradictory. It has generally been realized that the optimum depth is influenced by differences in soil and climate, but that the proper depth might vary with different varieties seems not to have been appreciated. The experiments referred to later, as well as many unpublished data showing the varying behavior of types when planted at different depths, indicate that it is unsafe and unscientific to generalize with respect to cultural factors without taking type, varietal, and even individual differences into account.

## MORPHOLOGY OF THE MAIZE SEEDLING

To explain this drought-resistant character, it will be necessary to discuss briefly the different parts of a maize seedling. (See fig. 1.) The primary root, or radicle, which is the first organ to emerge from the germinating seed, is soon followed by the shoot or plumule. Inclosing the shoot is the cotyledonary sheath, or coleoptyle, a tubular organ which is closed and pointed at the upper end. Between the base of the coleoptyle and the seed the axis is somewhat elongated. With seeds germinated in the laboratory this elongation is so slight that it might easily be overlooked. Nevertheless, this small organ has not escaped the notice of morphologists, and its nature has been the subject of much discussion. It has



been variously called "hypocotyl," "mesocotyl," and "epicotyl." By some it is held to be an internode, by others merely an elongated node.

The choice of a name for the organ depends on the interpretation of the homologies of the other parts of the embryo, particularly as to what is considered as constituting the cotyledon. If the sheath, or coleoptyle, be thought of as the cotyledon, the most appropriate name would be hypocotyl. Although this interpretation was accepted by Richard (1811),<sup>1</sup> Hofmeister (1858), and Sachs (1875), there seems to be little evidence in its favor and it is summarily dismissed by other morphologists.

The two remaining views are as follows:

(1) The scutellum alone is the cotyledon, the epiblast (absent in maize) representing a second leaf and the coleoptyle a third. The elongated axis between the coleoptyle and scutellum is thus considered an

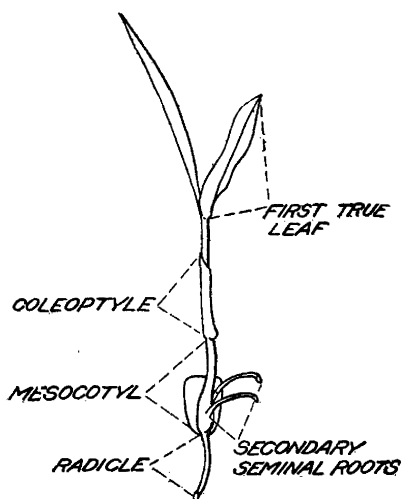


FIG. 1.—Diagram of seedling maize plant, giving terminology of parts.

internode and is then given the name "epicotyl." Among the supporters of this hypothesis are the following: Warming (1879-80), Hackel (1887), Bruns (1892), Van Tieghem (1897), and Holm (1908-9).

(2) All these organs, scutellum, epiblast, and coleoptyle, are viewed as parts of a more highly specialized cotyledon, in which case the term "mesocotyl" is applied to the portion between the coleoptyle and scutellum. With various modifications this last interpretation is adopted by Van Tieghem (1872), Hagelmaier (1874), Klebs (1881), Schlickum (1896), Čelakovský (1897), and Goebel (1905).

Van Tieghem originally subscribed to the view that the coleoptyle was a part of the cotyledon, but as a result of further investigations abandoned that position and adopted a modification of the views of Warming to the effect that the mesocotyl and coleoptyle represent a metamer distinct from the scutellum. The epiblast he held to be a rudimentary second cotyledon. Van Tieghem carried this interpretation to its logical conclusion and adopted the view that the apparent similarity between the grasses and other monocotyledons did not represent homologies, but that the two groups were phylogenetically distinct. He further held, on the strength of anatomical differences, that the portion of the axis between the scutellum and the coleoptyle is in some grasses an internode and in others an elongated node. The evidence regarding the morphology of the mesocotyl appears so conflicting that a definite interpreta-

<sup>1</sup> For "Literature cited" see p. 301.

tion satisfactory to all morphologists seems very remote. With organs that pertain to the very beginnings of the plant, even the primary differentiation into root, stem, and leaves may not be complete, and to insist on a definite classification of these primitive organs may be idle.

Studies of seedlings of Hopi maize show that the mesocotyl may frequently develop up to lengths of 36 cm.,<sup>1</sup> and it has been possible to note a fact which appears thus far to have escaped notice—namely, that the mesocotyl may give rise to roots at any point on its surface—but these roots are threadlike and do not resemble the roots that arise from the nodes of the culm. They do, however, closely resemble the roots that arise from the radicle immediately below the seed. (See Pl. XXIX, fig. 1.) In grasses roots usually arise from nodes, not from internodes, and the presence of roots on this organ in maize distinguishes it sharply from subsequent internodes and is an argument in support of the interpretation that this intercalary growth, long though it is, is really a part of the cotyledon and may properly be termed a mesocotyl. A further reason for retaining the term “mesocotyl” is because the interpretation implied by its use permits more direct comparisons with other groups of monocotyledonous plants, where the organ sheathing the plumule seems undoubtedly to be a part of the cotyledon.

From observations upon many varieties of maize it has become apparent that when a grain of corn germinates in the ground this usually insignificant organ is of vital importance to the life of the plant, for it is through the elongation of the mesocotyl that the shoot is enabled to reach the surface. So long as the seedling remains below ground, away from light, the mesocotyl will continue to elongate until it reaches a maximum length, which we have found to differ in different varieties, but which seems reasonably constant within the variety. As the mesocotyl elongates, the coleoptyle, with its firm, sharp point, is pushed upward through the soil. As soon as the coleoptyle emerges from the soil, the elongation of the mesocotyl ceases, and elongation of the internode bearing the first true leaf begins, forcing open the coleoptyle.

If the seed is planted so deep that the maximum elongation of the mesocotyl, which in anatomical structure shows a striking relation to the radicle, fails to bring the coleoptyle to the surface, the task of penetrating the soil and reaching light devolves upon the first true leaves. In comparison with the sharp coleoptyle, these leaves are but poorly adapted for forcing their way through the soil, and if the tip of the coleoptyle stops more than a few centimeters below the surface these leaves usually crumple and never reach the light.

In the varieties of maize commonly grown we have been unable to force the mesocotyl to a length greater than 10 cm., while in the Hopi and Navajo varieties this usually minute organ has in our experiments frequently reached the enormous length of 25 or even 30 cm.

---

<sup>1</sup> In *Euchlaena* also the mesocotyl may reach a length of 28 cm. Van Tieghem gives 3 cm. as the maximum length of this organ in grasses.

## GERMINATION OF NAVAJO MAIZE

It has been frequently stated that the Navajos, like their neighbors, the Hopi and Zunis, plant maize at unusual depths, 15, 30, and even 45 cm. having been reported. Since planting at such depths is known to be impracticable with other varieties, experiments were planned to test the ability of the Navajo maize<sup>1</sup> to pierce the soil. A representative experiment is here reported. A box 70 cm. long, 33 cm. wide, and 34 cm. deep was sunk in the ground. A quantity of sandy-loam soil sufficient to fill the box was slightly moistened and carefully sifted. At one end the box was filled to within 1 cm. of the top, the soil sloping in a straight line to within 1 cm. of the bottom at the other end.

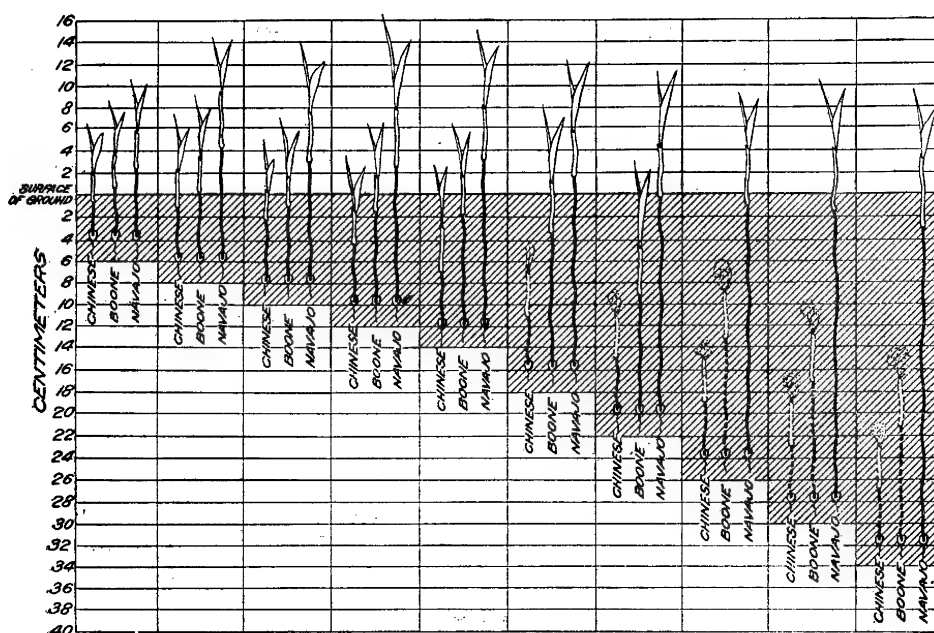


FIG. 2.—Diagram showing the average size of seedlings of Chinese, Boone County White, and Navajo maize planted at different depths.

Five seeds each of Navajo, Boone County White, and Chinese maize were placed in a row transverse to the inclined surface of the soil, 2 cm. from the top of the box. A similar row was planted at a depth of 4 cm. from the top, and so on at the following depths: 6, 8, 10, 12, 16, 20, 24, 28, and 32 cm. The box was then filled with the soil and struck off level with the top. The seeds germinated promptly, and when the most advanced seedlings had reached a total height of about 60 cm. the plants which appeared above the surface were dug up, and the mesocotyl and coleoptyle were measured. (See Table I and fig. 2.)

<sup>1</sup> In the fall of 1912 Messrs. Walter T. Swingle and Karl F. Kellerman visited the region about Shiprock, N. Mex., in the Navajo Reservation and secured specimen ears of the maize grown by the Navajos. This collection was kindly placed at the disposal of the writer. Additional seed was later secured through the courtesy of Mr. William T. Shelton, Indian agent at Shiprock.

TABLE I.—Average measurements of seedlings of Chinese, Boone County White, and Navajo maize planted at different depths.

Depth.	Chinese.			Boone County White.			Navajo.		
	Coleop- tyl.	Meso- cotyl.	Coleop- tyl and meso- cotyl.	Coleop- tyl.	Meso- cotyl.	Coleop- tyl and meso- cotyl.	Coleop- tyl.	Meso- cotyl.	Coleop- tyl and meso- cotyl.
<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>
2	2.3	2.3	4.6	3.7	3.2	6.9	5.5	5.0	10.5
4	2.5	3.5	6.0	3.1	4.9	8.0	4.3	6.5	10.8
6	2.8	5.0	7.8	3.4	6.1	9.5	5.2	10.2	15.4
8	2.5	5.8	8.3	2.8	7.4	10.2	4.9	11.0	15.9
10	3.2	5.8	8.9	3.1	8.6	11.7	5.6	12.2	17.8
12	4.0	5.2	9.2	3.4	10.4	13.8	5.0	15.1	20.1
16	.....	.....	.....	4.6	12.4	17.0	4.3	17.5	21.8
20	.....	.....	.....	4.5	10.9	15.4	4.7	19.7	24.4
24	.....	.....	.....	.....	.....	.....	5.2	23.0	28.2
28	.....	.....	.....	.....	.....	.....	5.6	26.5	32.1
32	.....	.....	.....	.....	.....	.....	6.5	29.0	35.5

Twelve cm. was the greatest depth from which seedlings of the Chinese variety appeared at the surface. Seedlings of Boone County White appeared from all depths up to 20 cm., while plants of Navajo maize appeared from all plantings, including the very deepest, 32 cm.

There were numerous instances in which the combined length of the mesocotyl and coleoptyle was less than the depth at which the seed was planted. This, of course, means that the upper layers of the soil were penetrated by the true leaves. The maximum depth of soil thus penetrated by the true leaves of the plants of the Chinese variety was 5 cm. One plant of Boone County White maize forced its leaves through 8 cm. of soil. In all of the Navajo plants the coleoptyle reached the surface.

The extent to which the seedlings of the Chinese and Boone County White varieties were able to penetrate the soil by means of the true leaves was doubtless much greater in the carefully prepared soil of the experiment than it would be under field conditions, where any slightly compacted lump of soil would deflect the tender leaves and cause them to crumple. On the other hand, many seedlings failed to come up where there was less than 2 cm. between the top of the coleoptyle and the surface of the ground. The results clearly show that the coleoptyle is the proper organ for penetrating the soil, and where this office devolves upon the leaves there will be many plants that fail to reach the surface.

It has been observed in many field plantings that the spatulate first leaf, formerly called the cotyledon, is the first evidence of the germinating plant. When this occurs in any considerable proportion of the plants, it is safe to assume that the seed has been planted too deep for the best results.

The three types of maize used in the box experiment were also planted in the field. Four seeds of each of the varieties were planted as follows: At the surface and at 5, 10, 20, 30, and 40 cm. below the surface. The greatest depth from which plants of the Chinese variety reached the surface was 10 cm., that of the Boone County White was 20 cm., while that of the Navajo was 30 cm.

The seeds planted at the surface were naturally the first to appear, but on June 17, one month after planting, the largest of the Chinese variety were those from a depth of 5 cm., while the largest plants of both the Boone County White and the Navajo maize were from the 10-cm. depth. On July 11 the plants that came up from a depth of 10 cm. were the tallest in all the varieties, including the Chinese, and to the end of the season this appeared the most favorable depth for the Chinese and Boone County White varieties. With the Navajo, however, the plants from a depth of 20 cm. had equaled those from the 10-cm. depth before the end of July, and from that time the plants from the 20-cm. planting continued to make the most rapid growth, as though this depth represented the most favorable condition for the Navajo variety.

#### DESCRIPTION OF ROOT SYSTEM

We have observed further that the root systems of the Navajo, Hopi, and Zuni varieties differ from those of the other varieties; the roots of their seedlings extend to a greater depth, and there is only a single root arising from each seed, while in the seedlings of the Chinese and Boone County White varieties the roots are shorter and more numerous.

The roots of maize are of two kinds: Those that arise from the embryo or seed, called "seminal roots," and those produced from the nodes of the plant. Of the latter class those that arise from the nodes above the ground are often called "brace roots" or "aerial roots." In the varieties commonly grown in the United States there are, in addition to the primary root, or radicle, from two to six additional roots that arise from the base of the cotyledon. These secondary seminal roots, though appearing somewhat later, usually equal or exceed the radicle in size. In the Pueblo varieties of maize these secondary seminal roots have been absent in all seedlings thus far examined, the radicle being the only root arising from the seed. (See Pls. XXIX and XXX, fig. 2.)

#### FIELD STUDIES OF PUEBLO VARIETIES OF MAIZE

In September, 1913, opportunity was afforded for a visit to the Zuni, Navajo, and Hopi Indian Reservations of Arizona and New Mexico. It was thus possible to form some idea of the agricultural significance of the peculiar habits of germination of this type of maize.

The value of deep planting made possible by the greatly elongated mesocotyl was obvious. In the localities selected by the Indians for

planting maize the soil is sandy, and in the absence of spring rains the surface layers are, of course, very dry. (See Pl. XXXI, figs. 1 and 2.) The seed, to germinate at all, must be planted deep enough to be in contact with the moist soil. In Navajo fields near Tohatchi, N. Mex., plants were dug up, and the remains of seeds were found at depths ranging from 13 to 18 cm. below the surface. Similar depths were found in a Zuni field near Black Rock, Ariz. (See Pl. XXXI, fig. 1.) In a Hopi field at Polacca, Ariz., near the First Mesa, where the conditions are extreme, the seed had been planted at a depth of 25 cm. (See Pl. XXX, fig. 1.) It thus appears that there is no fixed depth for planting, the custom being to plant deep enough to place the seed in moist soil. If the seed were planted at ordinary depths, germination might be delayed until the latter part of June or the first of July, at which time the rains usually occur; or if the seeds germinated as a result of one of the occasional showers occurring in May, the plants would die from subsequent desiccation.

Like the long mesocotyl, the simple radicle of the Pueblo varieties of maize may be looked upon as an adaptation to the extreme conditions that exist where these types are grown. For six or eight weeks after planting, no rain can reasonably be expected, and during this time the moisture is constantly receding from the surface. By concentrating the energy of the seedling into a single root the latter is forced to greater depths and consequently kept in moister soil than would be the case were a number of seminal roots developed.

Under ordinary conditions, where moisture is distributed through the entire seed bed, the seminal roots become of little importance as soon as the seedling is established and nodal roots have developed. If a half-grown or nearly mature corn plant is carefully dug up, the seminal roots and traces of the seed can still be found, but they are usually dry and shrunken and are obviously of little use to the plant. This was also the condition found in Navajo and Zuni maize fields, though the seminal root was more strongly developed than in the eastern varieties. (See Pl. XXIX, fig. 2.) But in the more extreme conditions existing in the fields near the Hopi villages, where the seeds were planted deeper, it was found that the seminal roots were relatively much larger and were still alive and fresh, making it apparent that they retain their function of supplying moisture and are able to play an important part during the entire life of the plant.

In one Hopi field at the base of the First Mesa the hills of maize were planted about 20 feet apart, with from 10 to 20 plants in a hill. The soil was apparently pure sand washed down by the winter rains and entirely destitute of vegetation other than the planted maize. An average hill dug up in the field was found to contain 15 plants ranging from 60 to 90 cm. in height. (See Pl. XXX, fig. 1.) The remains of the seeds were found at 25 cm. from the surface, and from each seed there

descended a single large seminal root. (See Pl. XXX, fig. 2.) These seminal roots were traced to a depth of 35 cm. and extended even farther down. They were still fresh and densely covered with fine branches. This mass of 15 seminal roots, while less in volume than the nodal roots arising near the surface, was apparently playing an important part in the support of the plants. The mesocotyls connecting the seminal roots with the plants above, while dry on the outside, were filled with live tissue quite unlike the dry and shrunken mesocotyls found in plants of similar age grown under more favorable conditions.

When planted by the Indian methods, the Hopi and Navajo varieties of maize have been found superior to the more improved eastern varieties for these very dry regions. At the time of our visit there was a small field near Keams Canyon that had been planted by eastern methods. The plants were in rows and thinned to one stalk to the hill. There had evidently been a fair germination, but the plants had died without reaching maturity and had produced no seed. At the same time, in the nearest Indian fields at Polacca the plants were dark green and maturing a fair crop, though the season was said to have been unusually dry. (See Pl. XXXI, fig. 3.)

Even under irrigation the somewhat larger strains grown by the Navajos have been found to compare very favorably with eastern types. Several acres of Navajo maize were seen at Shiprock, N. Mex., under irrigation. The fields were very uneven, apparently the result of alkali, but in the better portions the yield was good. The plants were standing about 2 feet apart in the row, the rows 4 feet apart, and nearly every plant was bearing from two to four fair-sized ears. (See Pl. XXXII.)

The ears from 36 plants, representing a number of distinct types, were collected. The 36 plants bore in all 94 ears, weighing 37.6 pounds, an average of 15.2 ounces per plant. The plants producing these ears averaged only a little over 5 feet in length.

#### CONCLUSIONS

Throughout the western part of the Great Plains area the difficulty of securing uniform germination is a serious obstacle to the growing of maize. With the varieties commonly grown, if the seed is planted at the customary depth, many seeds fail to germinate from insufficient moisture; if planted deep enough to come in contact with moist soil, the plants may fail to reach the surface.

The agricultural Indians of the Southwest have continued from pre-historic times to grow maize successfully in regions where drought, and especially the absence of spring rains, makes it much more difficult to start the crop than in the Great Plains. A study of the varieties grown by the Hopis and other agricultural Indians shows that these varieties possess two special adaptations: (1) A greatly elongated mesocotyl that permits deep planting and (2) the development of a single large radicle

that rapidly descends to the moist subsoil and supplies water during the critical seedling stage.

This indigenous type of maize seems to have attracted little attention, perhaps because it has been included in the popular mind with a series of inferior varieties commonly known as "squaw corn." But the Pueblo Indians of Arizona and New Mexico have strains sufficiently productive to compare favorably with improved varieties even when grown under irrigation. The peculiar adaptations of this type definitely indicate its value for the semiarid regions and warrant experiments to determine the possibility of its utilization.

#### LITERATURE CITED

BRUNS, ERICH.

1892. Der Grasembryo. *Flora*, Jahrg. 76, p. 1-33.

ČELAKOVSKÝ, L.

1897. Über die Homologien des Grasembryos. *Bot. Ztg.* Jahrg. 55, p. 141-174.

GOEBEL, K. E.

1905. *Organography of Plants*. Pt. 2, Oxford, p. 416.

HACKEL, EDUARD.

1897. Gramineae. Engler, Adolf, and Prantl, K. A. E., *Die Natürlichen Pflanzenfamilien*. T. 2, Abt. 2, p. 10.

HEGELMAIER, FRIEDRICH.

1874. Zur Entwicklungsgeschichte monokotyledoner Keime nebst Bemerkungen über die Bildung der Samendeckel. III. *Bot. Ztg.* Jahrg. 32, col. 661.

HOFMEISTER, WILHELM.

1858. Neuere Beobachtungen über Embryobildung der Phanerogamen, *Jahrb. Wiss. Bot.* [Pringsheim] Bd. 1, p. 154.

HOLM, THEODOR.

1908-9. Observations on seedlings of North American Phaenogamous plants. *Ottawa nat.* v. 22, p. 165-174, 1908; p. 235-244, 1909.

KLEBS, GEORG.

1885. Beiträge zur Morphologie und Biologie der Keimung. *Untersuch. Bot. Inst. Tübingen*, Bd. 1, p. 536.

RICHARD, L. CL.

1811. Analyse botanique des embryons Endorhizes ou monocotylédonés, et particulièrement de celui des Graminées. *Ann. Mus. Hist. Nat. [Paris]*, t. 17, p. 223-251; 442-487.

SACHS, JULIUS.

1875. *Text-book of Botany*. Oxford, p. 541.

SCHLICKUM, AUGUST.

1896. Morphologischer und anatomischer Vergleich der Kotyledonen und ersten Laubblätter der Keimpflanzen der Monokotylen. *Stuttgart*, p. 56. (*Bibliotheca Bot.* Heft 35.)

VAN TIEGHEM, PHILIPPE.

1872. Observations anatomiques sur le cotylédon des graminées. *Ann. Sci. Nat. Bot.* s. 5, t. 15, p. 236-276.

1897. Morphologie de l'embryon et de la plantule chez les graminées et les cypéracées. *Ann. Sci. Nat. Bot.* s. 8, t. 3, p. 259-309.

WARMING, EUG.

1879-80. Forgreningen og Bladstillingen hos Slaegten *Nelumbo*. [Footnote.] *Vidensk. Meddel. Naturhist. Forening. Kjøbenhavn*, p. 446-448.



## DESCRIPTION OF PLATES

PLATE XXIX. Fig. 1.—A seedling of Hopi maize with mesocotyl 18 cm. long. The seed was planted in sand 20 cm. below the surface. There is a single seminal root with threadlike branches similar to those arising from the mesocotyl. The first nodal roots have begun to form at the base of the coleoptyle. One-half natural size.

Fig. 2.—The root system of a plant of Zuni maize dug from a field near Zuni, N. Mex., showing the well-developed, single seminal root and the comparatively feeble nodal roots. Natural size. The field from which this plant was dug is shown in Plate XXXI, figure 1.

XXX. Fig. 1.—A hill of Hopi maize containing 15 plants grown under conditions of extreme drought at the base of the First Mesa near Polacca, Ariz. The ears can be seen borne at the surface of the ground.

Fig. 2.—A plant of Hopi maize. One of the smaller plants from the hill shown in figure 1. The remains of the seed are scarcely visible at the sharp bend of the mesocotyl, 25 cm. below the surface of the ground.

XXXI. Fig. 1.—A field of Zuni maize near Zuni, N. Mex. One of the hills near the center containing but a single plant shows a relatively large ear borne at the surface of the ground.

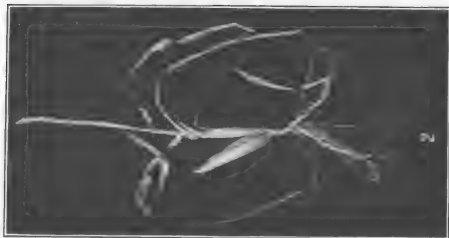
Fig. 2.—A hill of Zuni maize in the field shown in figure 1. Note the large ears borne near the surface of the ground.

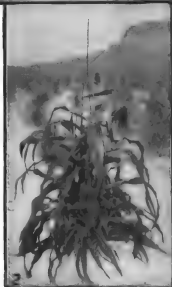
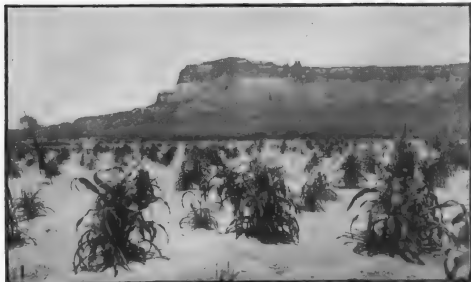
Fig. 3.—A hill of Hopi maize making luxuriant growth under conditions of extreme drought. Note the manner in which the low-spreading plants shade the ground. Polacca, Ariz.

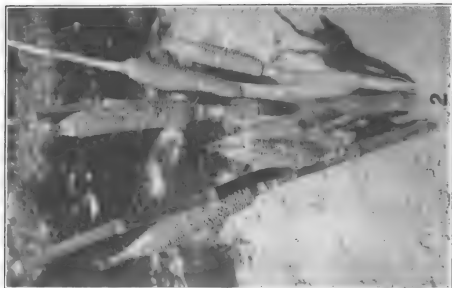
XXXII. Fig. 1.—A single plant of Navajo maize grown under irrigation at Shiprock, N. Mex.

Fig. 2.—The basal portion of the plant of Navajo maize shown in figure 1, with leaves and husks removed. The ears from this plant after drying weighed 2 pounds.









## SOME DISEASES OF PECANS

By FREDERICK V. RAND,

*Scientific Assistant, Fruit-Disease Investigations, Bureau of Plant Industry*

### INTRODUCTION

The pecan, *Carya illinoensis* (Wang.) K. Koch,<sup>1</sup> is an indigenous tree of the hickory group, which has long been famous for the excellent quality of its fruit. From the time when the earliest settlers first gathered the nuts from native forest trees the pecan has been growing steadily in favor.

Until recently the entire supply has come from the wild forest trees and from a comparatively few, more or less isolated seedling orchards. During the last 15 years, however, artificial propagation by budding and grafting has gradually assumed a commercial importance until at the present time a large number of excellent horticultural varieties are available. These are being planted on a large commercial scale and through an ever-widening range.

The pecan is found native on low, rich ground in the neighborhood of streams from the valley of the Mississippi River in Iowa through southern Illinois and Indiana, western Tennessee to central Alabama and Mississippi, western Louisiana through Arkansas and Missouri to southeastern and western Kansas, eastern Oklahoma, and the valley of the Concho River, Tex. It is also found in some of the mountain regions of Mexico. As a native tree the pecan is most abundant and attains its largest size in southern Arkansas, eastern Oklahoma, and middle to eastern Texas.<sup>2</sup> As a cultivated tree, however, it is by no means confined to the sections above enumerated. Plantings of greater or less extent have been made in Virginia, North Carolina, South Carolina, Georgia, Florida, New Mexico, California, Oregon, and Washington, with small experimental plantings in several other States.

Notwithstanding the highly colored statements of some of the early promoters of pecan culture, this tree, like all of our cultivated fruit trees, has its insect and fungous enemies. Possibly they would form a shorter list than would those of some of our common fruits, but they are none the less real and important, for, whenever a plant is brought under cultivation or taken out of its native range, new diseases and new problems with old diseases are sure to follow.

Other things being equal, the larger the number of individuals of a host species growing in a given area the greater the chances any particu-

---

<sup>1</sup> Synonyms: *Carya olivaeformis* Nutt.; *Hicoria pecan* Brit.; *Juglans pecan* Marsh.

<sup>2</sup> Sargent, C. S. *Manual of the Trees of North America*. Boston, 1905, p. 134.

lar parasite has of successfully reproducing itself from season to season, and consequently the more general and severe will be its injury over that area. Thus, a disease occurring occasionally or with but slight injury upon more or less isolated host individuals may under conditions of close orchard planting assume an entirely different aspect, becoming more nearly seasonal in its occurrence and causing a much greater percentage of injury. A large part of the assumed difference in injury by a disease under native and under orchard conditions is, however, often merely psychological. In orchard culture the ideal sought is a thrifty growth and abundance of high-grade fruit for every tree planted. Any deviation from this ideal is quickly noted by the grower; whereas little consideration is given to the facts that under native conditions large numbers of individuals succumb to disease for every one that persists and reaches maturity and that careful observations and comparisons are seldom made with those which do reach maturity.

Nevertheless, the general fact remains that well-known diseases are often more destructive under orchard conditions. Further than this, diseases of hitherto unknown occurrence upon a particular host may suddenly make their appearance. Some of these may have been present but previously unnoticed, while others may be actually new to the host. They are often brought to a locality with the introduction of new plants, and with the widening of the range of a host the diseases of related plants will be encountered sooner or later. Furthermore, a parasite is often more destructive when brought to a new locality, either because of the absence of its former enemies or because of other conditions more favorable to its growth and reproduction in the new environment.

It has long been known that where a considerable number of plants or animals are exposed in a similar way to the attacks of a parasitic disease more or less difference will be noted in their behavior toward the disease. In many cases some individuals will be found which seem to be entirely immune, others which are very susceptible to attack, and still others with varying grades of immunity or susceptibility between two extremes. In localities favorable to the growth and spread of a disease this condition works for the general benefit of the species attacked. Those individuals least susceptible to injury will be most successful in reproducing themselves, and thus a more or less immune race will be developed. On the contrary, if a race has arisen amid conditions unfavorable to the development of a particular disease, or in its entire absence, growth and reproduction will have taken place with little or no relation to the disease. If such a race is exposed to the disease, it is probable that a large percentage of its individuals will be found to be susceptible.

These relations between host and parasite, though only a few among many, may at least serve to indicate the extreme complexity of all prob-

lems having to do with living things. Partly because of this complexity most problems of disease control are problems of "better and worse" rather than of "good and bad," for very few varieties prove to be absolutely immune, and very few artificial methods of control are entirely effective.

The present paper deals only with certain distinct and more or less troublesome fungous and bacterial diseases of pecans.<sup>1</sup> For the most part these studies were carried on during the years 1911 and 1912.

#### NURSERY-BLIGHT

[Caused by *Phyllosticta caryae* Peck]

#### HISTORY AND DISTRIBUTION

Nursery-blight is one of the worst known diseases of the pecan to affect nursery seedling trees. However, in spite of the fact that young trees are often defoliated from this cause by midsummer, no definite investigation has hitherto been carried out and published, so far as could be ascertained. This may be due partly to the fact that the pecan nursery business is of comparatively recent origin and partly to the obscurity of the causal fungus.

The distribution of this disease has been found to correspond very closely with that of the pecan scab and the brown leaf-spot. Affected specimens have been received from most of the pecan-growing States, and personal observations have further demonstrated its presence at Petersburg, Va.; Orangeburg, Summerton, and Charleston, S. C.; Albany, De Witt, Hardaway, Baconton, Thomasville, and Cairo, Ga.; Tallahassee, Newport, Monticello, Glen St. Mary, Jacksonville, St. Augustine, Palatka, and Belleview, Fla.; New Orleans, La.; and at San Antonio, Boerne, Kerrville, Waco, and San Saba, Tex. Strains of the fungus obtained from as widely separated points as Florida and Texas have been similar in cultural characters and have caused the same symptoms upon artificial inoculation, thus demonstrating the disease in both cases to be of the same origin. Wherever observations have been made the disease has for the most part been found to affect young trees, and by far the greatest injury has been to the 1 and 2 year old nursery stock.<sup>2</sup> Mature trees are seldom seriously injured.

<sup>1</sup> No discussion of the scab, a serious disease of pecans, is included in the present paper.

<sup>2</sup> A very effective control of the nursery-blight with Bordeaux mixture was obtained in two different localities during the season of 1911, and there appears to be little reason to doubt that it will prove efficacious in other localities and seasons. The quantity of spray material used and the cost of application under nursery conditions are small, and it is thought that the increase in size and vigor, together with better conditions for budding, will amply repay the small cost in material and labor necessary for the treatments. It is obvious that the first application should be made before the disease has gained much headway in the spring. Three to five subsequent applications may then be given at intervals of three to four weeks, according to the season.



## SYMPTOMS OF THE DISEASE

So far as has been observed nursery blight affects only the leaf blade, but infections occur from early spring well on through the season, so that under conditions favorable to the development of the disease the young trees have little opportunity for growth. Generally the first indications of infection appear in the form of minute roundish spots, which are dark reddish brown on the upper leaf surface and blackish on the lower. (Pl. XXXVII, Fig. C.) These slowly increase in size until a diameter of 2 to 5 mm. is often reached in the individual spots. With increase in size the center of the spot on the upper surface assumes an ashen-gray color, which is usually bordered with reddish brown, while the lower surface remains black throughout or with an occasional tiny ashen-gray spot in the center of this dark-colored area. (Pl. XXXVII, Fig. I.) The gray color in both cases is caused by a raising of the epidermis, thus leaving an air space between it and the tissues

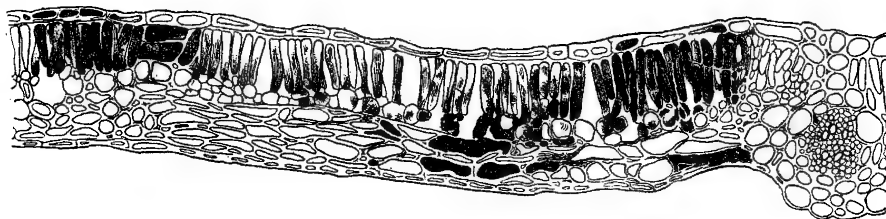


FIG. 1.—Cross section of pecan leaf recently infected with the nursery-blight fungus (*Phyllosticta caryae* Peck) from pure culture.  $\times 260$ .

below. The leaves are often considerably peppered with these spots, and by their coalescence larger areas are often involved. Very frequently the spots elongate and coalesce along the midrib and larger veins, thus giving a very characteristic appearance. The parenchyma cells and vascular bundles are often killed and discolored over large areas. Whenever the vascular tissue becomes involved to any great extent the supply of water is cut off from below and the leaf soon dries up and falls. Figure 1 shows the microscopical appearance of the diseased cells in a recently infected leaf.

## MYCOLOGICAL AND PATHOLOGICAL STUDIES

## ISOLATION OF THE FUNGUS

Rough microscopical examination of a considerable range of diseased material disclosed no fungous or bacterial form which was at all constantly associated with the symptoms. Occasionally a tiny thin-walled pycnidium was encountered, but no spores were found and usually no fungous mycelium or fruiting body of any kind. Cultures made during 1910 and 1911 from material several days old gave only saprophytic fungi as shown by the negative results of all the inoculation tests.

Experimentation had already shown that the disease was readily controllable by Bordeaux mixture, and hence it was thought highly probable that it was of parasitic origin. Consequently, in the summer of 1912, materials for making cultures were taken directly into the field with the idea of locating the cause, if possible, by any of the ordinary methods of isolation. Leaves showing very recent infection were taken from the highest parts of the trees where there was little or no spattering from the soil. These leaves were placed in sterile Petri dishes and taken to the temporary laboratory, where the tiny spots were cut out at once with sterile scissors and transferred by the ordinary poured-plate method to Petri dishes of corn meal and synthetic agar.<sup>1</sup> After 24 to 48 hours colonies became visible which had evidently originated from the diseased areas, and their appearance was quite uniform in all the cultures, except in a few cases where contaminations had entered. Transfers were then made to tube cultures. In this way strains of the fungus were obtained from Monticello, Fla., from Albany, Ga., and from San Antonio, Waco, and San Saba, Tex.

#### INOCULATIONS

Circumstances connected with field travel prevented the making of any inoculation tests during the summer of 1912, but the following summer and winter trials were carried out upon potted seedling trees in the greenhouse. The trees were sprinkled, inoculated from pure cultures, and covered after inoculation for several days with bell jars. Three strains of the fungus were used in this work: One from Texas, one from northern Florida, and a strain reisolated from an artificially infected leaf.

EXPERIMENT NO. 1 (Oct. 8, 1912).—The young leaves on four trees were inoculated from 1½-months-old, nonsporiferous synthetic-agar cultures (Florida strain 122), the slimy mycelial mass being smeared over portions of both leaf surfaces. These four trees and two moistened but uninoculated check trees were left under bell jars for five days. After a week small dark-brown specks were noted over the inoculated areas. In three weeks these spots were 1 to 2 mm. in diameter and in every way similar to natural infections. The check trees remained uninjured.

EXPERIMENT NO. 2 (Nov. 9, 1912).—The young leaves on three seedlings and the matured leaves on two others were inoculated as above only from 3-weeks-old, sporiferous corn-meal-agar cultures (Florida strain 122). The five inoculated and five check trees were left under the bell jars for three days. Observation after two weeks showed the production of small, roundish, dark-brown specks, which at three weeks had become 1 to 3 mm. in diameter with small ashen-gray areas in the center. The lower

<sup>1</sup> SYNTHETIC AGAR.—(1) 1,500 c. c. of distilled water and 36 grams of agar. Cook in double boiler for one hour at 15 pounds pressure.

(2) 500 c. c. of distilled water, 200 grams of dextrose, 40 grams of peptone, 20 grams of ammonium nitrate, 5 grams of magnesium sulphate (crystals), 10 grams of potassium nitrate, 5 grams of potassium acid phosphate ( $K_2HPO_4$ ), and 0.2 gram of sodium chlorid.

Boil in double boiler for 30 minutes, add agar and cook for five minutes. Restore to volume, titrate, cool to 60° C., and add whites of two eggs. Cook to coagulate eggs, filter, tube, and sterilize.

This formula is modified from that given by Francis Darwin and E. Hamilton Acton in their *Practical Physiology of Plants*, ed. 3, 1901, p. 68.

surface of the infected areas was almost black. Infection had taken place upon all the leaves inoculated, while none of the check trees showed any signs of the disease.

EXPERIMENT NO. 3 (Dec. 7, 1912).—The mature leaves of four seedlings were inoculated from 3-weeks-old, sporiferous corn-meal-agar cultures (Florida strain 122), and those of three other seedlings from nonsporiferous synthetic-agar cultures of the same age and strain. The seven inoculated plants and four checks were kept under bell jars for three days. Observations at two weeks showed the leaves of the first set with tiny dark-brown specks scattered over the inoculated areas and with some of the spots beginning to show the grayish centers. The leaves inoculated from the synthetic-agar cultures were similar, but not quite so far advanced. The check trees all remained uninjured.

EXPERIMENT NO. 4 (Dec. 7, 1912).—The mature leaves of three seedlings were in like manner inoculated from 3-weeks-old, sporiferous corn-meal-agar cultures (Texas strain 127). At the end of one week the spots were just becoming visible, and after two weeks the centers were turning gray on the upper surface, while the borders remained the typical dark brown. There were no evidences of the disease on the two check trees. All five trees had been covered with bell jars for the first three days.

EXPERIMENT NO. 5 (Dec. 18, 1912).—Two trees were inoculated from corn-meal-agar cultures isolated from one of the trees of experiment No. 3 (strain 163). Typical infections appeared at five to seven days, and these gradually increased in size for three weeks, finally taking on the grayish center and dark reddish brown border above, with the color almost black below. The check trees remained healthy.

EXPERIMENT NO. 6 (Dec. 18, 1912).—The mature leaves of three seedlings were inoculated from sporiferous corn-meal-agar cultures of two weeks' incubation (Florida strain 122). In this case the pycnidia were broken up in sterile distilled water and sprayed upon the leaves. The three check trees were sprayed with sterile distilled water, and all six trees were left under bell jars for three days. On removing the bell jars it was noted that tiny dark-colored specks were forming over much of the areas inoculated. These later proved to be the typical spots of the nursery-blight. No evidence of disease appeared on the check trees.

EXPERIMENT NO. 7 (Dec. 23, 1912).—The sporiferous pycnidia from young corn meal-agar cultures (Florida strain 122) were broken up in sterile distilled water and sprayed upon the upper and lower surfaces of the leaves of three seedling pecan trees, the leaves having previously been washed. Three days after inoculation sample inoculated and check leaves were collected. These were killed and bleached in alcohol, stained with eosin, and examined superficially under the microscope. The conidia themselves, being almost bacillar in size, could not be seen with the low power necessary in any such examination. However, here and there could be distinguished a very fine mycelial growth stained pale pink by the eosin, and in a number of cases hyphæ were clearly seen entering the leaf through stomatal pores or openings left by the breaking off of leaf hairs and resin glands. In one case the branching hypha could be followed some distance beneath the epidermis from the stoma through which it had entered. The check leaves showed no such fungous growth entering the leaf.

After a week an examination of the leaves left on the trees showed tiny dark-colored spots scattered over the inoculated areas, while at two weeks the typical grayish centers had developed. The check leaves were still without injury.

In the above detailed experiments the leaves of 24 pecan seedlings were inoculated at different stages of maturity and with three strains of the fungus. Every inoculation was successful, and in no case did any of the check trees show signs of the disease. These data seem to establish the parasitism of the fungus beyond any doubt.

From the facts that most of the infections occur within 2 or 3 feet of the soil surface, that such infections may take place through stomata and other openings in the epidermis, and that pycnidia are of rare occurrence upon the leaves while still attached to the tree, it seems very likely that the general development of pycnidia takes place upon the dead and decaying leaves after they have fallen to the ground and that most of the infection occurs through the spattering of spore-bearing material from the soil.

#### CULTURAL STUDIES

##### THERMAL TESTS

Four series of thermal tests were carried out, corn-meal-agar cultures being incubated for two to three weeks at temperatures ranging from 1° to 40° C. No change occurred at 1° or at 40°, while at 5° and 36° growth, where it occurred at all, was so small as to be scarcely discernible. The growth of the colony was extremely slow at 8°, but increased considerably in rate at 12°. At 14°, 16°, and 20° the rate was nearly the same, though with a very gradual increase toward the higher temperature. The optimum for the temperatures tested occurred at 30°, while at 32° growth was very similar to that at 12° to 14°. Incubation of two or three weeks at 37° to 40° invariably killed the fungus, no subsequent growth taking place when again held at optimum temperatures.

Thus, incubated in corn-meal-agar slant tubes, the fungus made at least some growth at temperatures ranging from 5° to 36° C. (41° to 97° F.); with a very gradual decrease in rate from the optimum (30° C. or 86° F.) downward, and a rather rapid decrease upward. The comparatively high optimum temperature, together with the wide range of effective growth at lower temperatures, will assist in explaining the extended and continuous period of infection observed under field conditions.

##### CULTURAL CHARACTERS

The more obvious characters of the fungus as grown upon a number of culture media are as follows:

**Beef-Agar Slant Tubes.**—The colonies are at first somewhat convex, pale ochreous in color, with slightly roughened but glistening surface, and without aerial mycelium. Later, the surface becomes much wrinkled, often presents a corallike growth in the older parts, and approaches a light Venetian red in color. A moderate production of pycnidia usually takes place in cultures 1 or 2 months old. Colonies often attain a diameter of 10 to 12 mm.

**Corn-Meal-Agar Slant Tubes** (Pl. XXXVII, fig. H).—Where little aerial mycelium is present, the colonies are at first about the same color and general appearance as in the young beef-agar cultures. The cottony aerial mycelium becomes a faint pinkish white and is often present in considerable luxuriance. The submerged parts sometimes give a pale-violet tinge to the agar, but little or no direct diffusion of color into the medium has taken place. Pycnidia are produced in abundance and range from 75 to 150  $\mu$  in diameter. At first they are a pale-ochreous color, but later change to dark brown or almost to black. Many cross connections between the hyphæ have

been observed, and swollen cells are commonly scattered here and there through the mycelium. Colonies often cover the slant, but unlike those on beef agar they are seldom much wrinkled.

**Corn-Meal Flasks.**—On this medium the colonies with 1 or 2 months' growth attain a diameter of 5 or 6 cm., and become deeply convoluted or wrinkled. The cottony aerial mycelium where present is similar in color to that on corn-meal agar, while the underlying pseudoparenchyma en masse takes on a yellowish burnt-sienna tinge. Pycnidia were not observed.

**Filter Paper.**—Growth on filter paper moistened with sterile distilled water gave small colonies of a pale-violet color and with or without a scant pinkish white aerial mycelium.

**Oxalic-Acid-Agar Slant Tubes.**—The colonies are raised to convex, pale ocherous around the margin and approaching a sepia brown throughout most of the central portion. With age the vegetable dye of the medium becomes bleached, so that the color of ordinary beef agar is finally assumed. No pycnidia were observed.

**Synthetic-Agar Slant Tubes**<sup>1</sup> (Pl. XXXVII, fig. G).—The colonies are very convex, with moist and glistening surface. The mycelial mass is extremely viscous, much convoluted, burnt sienna to brown in color, and with the drying out of the cultures assumes various shades of olive green, violet, brown, and reddish brown. Numerous cross connections between the hyphæ were noted, but no pycnidia have yet been developed on this medium.

#### MORPHOLOGY AND TAXONOMY

Several of the diseased spots from fresh material were killed in Carnoy's fluid, embedded in paraffin, and sectioned both vertically and horizontally in order to locate the course of the fungous growth within the tissues. The mycelium was found to be septate, very fine, and nearly or quite hyaline; and even in the stained vertical sections it was often distinguishable with difficulty. This readily explains the fact that examination of rough mounts from field material rarely gives any evidence of fungous growth within the leaf tissues. The mycelium was best located in the stained horizontal sections, where it could be distinctly seen ramifying through the intercellular spaces just above the lower epidermis and throughout the mesophyll tissue. (Fig. 2.) Where the spots involved the vascular tissue, the hyphæ were often seen extending immediately parallel to the vessels, the latter in such cases being dead and discolored. In many cases this intercellular mycelium had developed scattered swollen cells with large vacuoles, but thus far no definite formation of pycnidia has been observed upon artificially infected leaves. Upon field material, however, the tiny dark-colored fruiting bodies are occasionally encountered upon the upper leaf surface.

In 1887 a *Phyllosticta* occurring on *Carya alba* was described by Peck,<sup>2</sup> which from his description and an examination of type specimens, appears to be identical with the nursery-blight fungus. Peck's description is as follows:

<sup>1</sup> For the formula for preparing synthetic agar, see p. 307.

<sup>2</sup> Peck, C. H. Plants not before reported. 40th Ann. Rpt., N. Y. State Mus. Nat. Hist., 1886, p. 57. 1887.

*Phyllosticta caryae*, n. sp.—Spots large, irregular, often confluent, at first yellowish, then brown, sometimes becoming grayish in the center; perithecia minute, .004 inch broad, punctate, epiphyllous; spores irregularly elliptical, .0002 inch long, .00008 broad.

Living leaves of hickory, *Carya alba*, Piffard, August.

Several months afterwards Ellis and Everhart<sup>1</sup> described under the same name a *Phyllosticta* occurring on species of *Carya* at Newfield, N. J. On account of Peck's priority, the specific name of Ellis and Everhart's fungus was later changed by Saccardo to *caryogena*.<sup>2</sup>

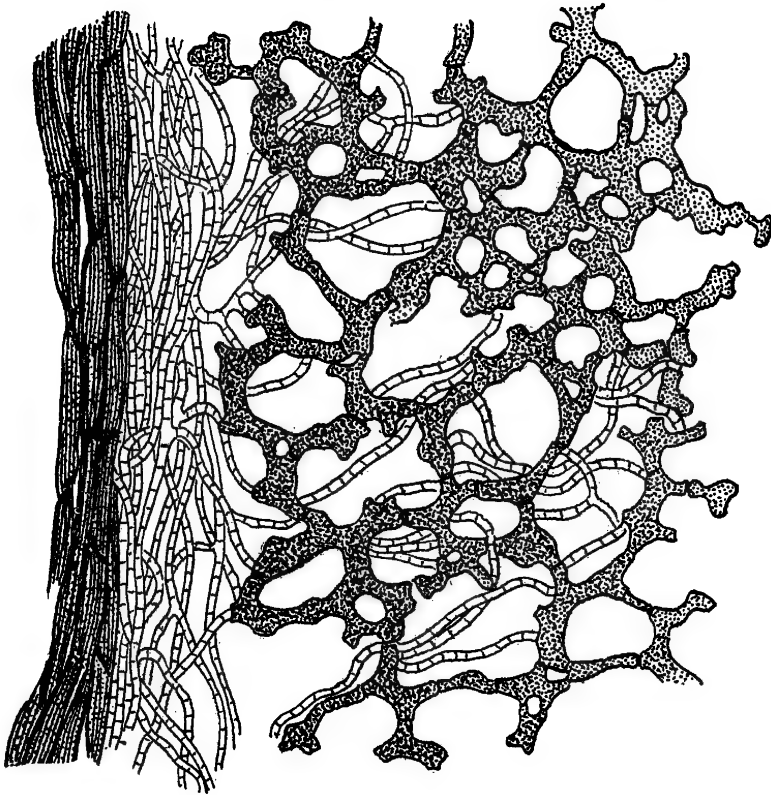


FIG. 2.—Horizontal section of leaf recently infected with the nursery-blight fungus in pure culture.  $\times 150$ .

After examination of Peck's material the two species were finally considered by Ellis and Everhart as identical, and the following description and statement was published:

*Phyllosticta Caryae* Pk. 40th Rep. 57. 1887.

*P. Caryae* E. & E. Journ. Mycol. 101. 1888.

*P. caryogena*. Sacc. Syll. 10:119. 1892.

Exsicc. Ell. & Evrht. N. A. F. 2155, 2677.

On various species of *Carya* from Maine to Kansas.

Spots large, irregular, often confluent, often acute at each end, with a nerve of the leaf running through the center, .5-1 cm. diam., yellowish at first, becoming brown,

<sup>1</sup>Ellis, J. B., and Everhart, B. M. New species of fungi from various localities. Jour. Mycol., v. 4, no. 10, p. 101, 1888.

<sup>2</sup>Saccardo, P. A. Sylloge Fungorum. v. 10, Patavium, 1892. p. 119.

with the margin darker. Perithecia epiphyllous, minute, lenticular, black-brown,  $100\ \mu$  broad. Sporules oblong or ellipsoid-oblong,  $5-8 \times 2-2.5\ \mu$ . The fungus is also found on old insect-galls on the same leaves. The 40th Rep. was given to the public in May, 1888. *P. Caryae* E. & E. was not published till October, 1888. *P. Caryae* Pk. and *P. Caryae* E. & E. are evidently the same<sup>1</sup>

The leaf spots upon the pecan assume the reddish brown color at a very early stage of development, though this is often preceded by a slight yellowing of the tissue at the point of infection. Furthermore, the grayish center is almost invariable in its appearance during the later stages. Individual spots have rarely been found by the writer to exceed 4 or 5 mm. in diameter, but by the coalescing of several initial infections diseased areas at least up to 8 or 10 mm. have frequently been observed.

The majority of the pycnidia have been found to vary but little from  $100\ \mu$  in diameter, but extremes of 50 to  $150\ \mu$  have been noted for mature pycnidia in culture. In the latter case they are usually much lighter in color than on the host, assuming macroscopically a tawny appearance. On the pecan leaf and occasionally in culture the fruiting bodies are dark brown to black.

Conidia as observed on this host have corresponded closely with Peck's fungus, ranging within the limits of 3.8 to 6 by 1.5 to  $2\ \mu$ . In other points also the pecan fungus corresponds closely with the two descriptions quoted above.

Thus, on account of the close relationship between the hosts and the many points of resemblance between the fungi and the disease symptoms, it seems best to consider the nursery-blight fungus as identical with *Phyllosticta caryae* Peck rather than to burden mycological literature with another name. At least this course should be followed until cultural and cross-inoculation work can demonstrate a specific difference.

#### BROWN LEAF-SPOT

[Caused by *Cercospora fusca*, emend. sp.]

#### HISTORY AND DISTRIBUTION

With the growth of the pecan industry the brown leaf spot has gradually been receiving more notice among orchardists. Since it is by no means as serious a trouble as the pecan scab, it has not merited the attention given the latter. No published record has been found, except a brief description of the fungus, and no work establishing the cause or demonstrating a method of control.<sup>2</sup> However, next to the pecan scab it is perhaps the worst and most generally distributed leaf-spot disease

<sup>1</sup> Ellis, J. B., and Everhart, B. M. The North American *Phyllostictas*. Vineland, N. J., 1900. p. 35.

<sup>2</sup> The brown leaf spot has occurred to a limited extent at points where spraying tests were being carried out on other pecan diseases and has been effectively controlled with three treatments of Bordeaux mixture.

affecting the mature trees and consequently has been considered worthy of investigation as well from a practical as from a mycological standpoint.

For several years specimens of leaves showing this disease have been received from widely different parts of the pecan-growing territory, while within the last two years the writer has made personal observations in the field over much of this region. From these observations and studies in field and laboratory it may definitely be said that the brown leaf-spot occurs in South Carolina, Georgia, Florida, Alabama, Louisiana, and Texas and that an exceedingly similar if not identical disease has in numerous instances been seen on other species of hickory. Furthermore, there is little doubt that its range is much greater than that above indicated, since it has been found in nearly every pecan section visited by the writer during the last three years.

Observations in several States during the past two years have shown very little difference in resistance to the disease among the different varieties. For example, in one orchard examined, containing 45 varieties of the pecan, the brown leaf-spot was so uniformly distributed that no appreciable difference in the amount of injury could be detected among the different varieties. From a number of such observations over a wide territory it may be safely assumed that little difference in resistance exists among the varieties now commonly planted.

#### SYMPTOMS OF THE DISEASE

The leaf blade is the only part of the tree known to be affected. (Pl. XXXVII, fig. A.) In ordinary seasons or when only a few spots occur, there is little or no appreciable injury, but occasionally under conditions very favorable to the progress of the disease partial defoliation may result. Infections occur from the early part of summer on until fall, and under proper conditions of moisture and temperature even well-matured leaves may develop the disease. Several days after infection (ordinarily 3 to 10) the condition becomes evident through the formation of a tiny dark reddish brown spot, which is usually somewhat angular in outline and bounded by the veins of the leaf. The spots from the earliest visible stages extend through the leaf tissue and appear about the same in form and color on both surfaces. The size increases gradually until the diseased areas often attain a diameter of 10 or even in some cases 15 mm. With increase in area the spot often loses its angular outline and the margin becomes more indefinite, while at the same time the center of the spot may in some cases assume a somewhat lighter reddish brown color with the darker brown as a border. Very often, however, the spots remain angular and with definite margin, though in such cases they seldom attain a diameter of more than 2 or 3 mm. Microscopical examination showed the cells within the affected areas to be dead, more or less opaque, and brownish in color. (Fig. 3.)



## MYCOLOGICAL AND PATHOLOGICAL STUDIES

## ISOLATION OF THE FUNGUS

Examination of a wide range of material during the last three years has invariably shown the same type of fungous growth and spore formation, while no other fungi have been found, except in the later stages of the disease. It was considered very probable that the fungus above mentioned was the cause of the diseased condition which it accompanied, and so on October 4, 1911, single spore cultures were started, using conidia from material collected August 29, 1911, at Baconton, Ga. Synthetic agar was used, and the germination was followed under the microscope from day to day. Growth was rather slow at first, but continued until at the end of a month colonies 5 to 15 mm. in diameter had been formed.

From one of the strains obtained in this way the first inoculation tests were made.

## INOCULATIONS

The inoculation work was carried out during the winter and spring of 1912 upon young seedling pecan trees in the greenhouse. The leaves to be used in the tests were moistened with water immediately preceding inoculation, and since no definite spore formation has taken

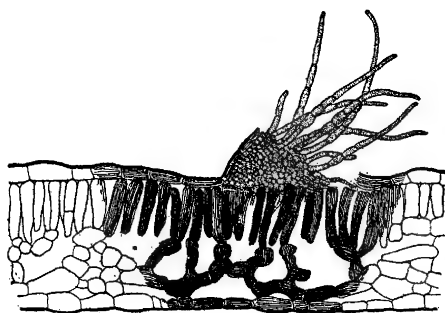


FIG. 3.—Cross section of a leaf infected with the brown leaf-spot fungus from pure culture.  $\times 250$ .

place in culture, bits of the mycelial growth were placed directly on the upper or lower sides of the leaves thus moistened. The small potted trees were then generally left for several days under bell jars, with slight ventilation at the base, to insure proper humidity for growth of the fungus. Check trees in each experiment were treated similarly, with the exception of the inoculation.

EXPERIMENT NO. 1 (Feb. 29, 1912).—The young leaves of two potted seedlings were inoculated from 3-weeks-old oxalic-acid and synthetic-agar cultures (strain 33), the first tree being covered with a bell jar and the second left open. Two check trees were placed under a bell jar. The inoculated and check trees were all sprinkled with tap water on the second and fifth days and the bell jars were removed on the latter date. At the end of two weeks most of the inoculated leaves on the first tree had developed small, reddish brown areas from mere angular flecks up to irregularly circular spots 1 mm. in diameter. Very little infection had occurred on the tree left uncovered after inoculation, but several distinct spots were noted. Later, many of the spots had increased in size up to 7 or 8 mm., with the development of tawny clusters of conidia visible to the naked eye upon the upper leaf surface. In no case did the check trees show signs of the disease.

EXPERIMENT NO. 2 (Apr. 16, 1912).—In a similar manner the tender leaves of a seedling were inoculated on both surfaces from a month-old corn-meal-agar culture (strain 33). This tree and the check were left under a bell jar for five days. Observation after a month showed a large number of the somewhat angular young spots up

to 1 mm. in diameter, but no spore formation had as yet occurred. After two months the spots were well scattered over the inoculated areas, and some of them had attained a diameter of 10 mm. The pale tawny conidial tufts were at this time very abundant on the upper surface. No infection had taken place on the check tree. After three months many of the smaller spots had coalesced to form reddish brown areas up to 20 mm. in diameter.

EXPERIMENT NO. 3 (Apr. 29, 1912).—The tender leaves of two seedlings were inoculated on both surfaces from 5-weeks-old corn-meal-agar cultures (strain 33). These and the two check trees were left under bell jars for six days, the leaves being sprinkled on the second and fourth days. At 10 days infection was just becoming evident, while at the end of one month all but one of the inoculated leaves were peppered with the more or less angular reddish brown spots. After six weeks the development of conidial tufts began to take place on the upper leaf surfaces.

EXPERIMENT NO. 4 (May 28, 1912).—The tender leaves of two seedlings were inoculated on the lower surface from a month-old synthetic-agar culture (strain 33) and these, with the single check tree, were covered with bell jars for six days. Observations after three weeks showed the typical spots of this disease up to 3 and in one case 6 mm. in diameter. The conidial tufts were just beginning to form. No infection occurred on the check tree.

EXPERIMENT NO. 5 (May 29, 1912).—The young leaves of two seedlings were inoculated from a month-old culture (strain 33) on sterile pecan wood, and the tree was left under a bell jar for several days. At the end of one month numerous somewhat angular reddish brown spots were evident on all the leaves, and these varied in size from mere specks to areas 10 or 15 mm. in diameter. After six weeks the development of conidial tufts had commenced. No infection occurred on the single check tree.

EXPERIMENT NO. 6 (June 7, 1912).—The rather mature leaves of two seedlings were inoculated on both surfaces from a 4-weeks-old synthetic-agar culture (strain 113, an isolation from the artificially infected leaves described in experiment No. 1). The air was hot and dry at this time, and hence the bell jars were left over these trees and the three checks for eight days. Observations at the end of two weeks showed the development of typical spots on all the inoculated leaves, and at one month the formation of conidial tufts had begun.

EXPERIMENT NO. 7 (3 p. m., May 23, 1912).—The tender leaves of a young tree of the Schley variety at Arlington Farm, Virginia, were inoculated on both surfaces from a 4-weeks-old prune-agar culture (strain 33). The day was cloudy, but the hot, dry weather of the following week prevented infection.

EXPERIMENT NO. 8 (2.30 p. m., June 7, 1912).—A second young Schley pecan tree at Arlington Farm was inoculated from a 4-weeks-old synthetic-agar culture (strain 113). The day was cloudy, and the leaves were covered with moistened cotton to further insure the growth of the fungus. The weather was rather hot and dry for several days afterwards, but this period was followed by a day or so of rain. Later observations showed a moderate number of the typical spots on the inoculated leaves, while the check leaves showed no signs of the disease.

EXPERIMENT NO. 9 (3 p. m., June 15, 1912).—In a similar manner the four to six young shoots of three Schley pecan trees at Arlington Farm were inoculated from 6-weeks-old corn-meal flask cultures (strain 33). In this case the shoots on one inoculated and one check tree were covered by heavy paraffined paper bags containing moist blotting paper to insure a high humidity around the inoculated leaves, while those on one check and two inoculated trees were left uncovered. Showers occurred on the two following days. Examination in the fall showed many of the typical spots developed on the inoculated leaves covered by the bags and on those of one tree left uncovered. There was no infection on any of the check trees.

EXPERIMENT NO. 10 (June 15, 1912).—The young leaves of one potted seedling and the mature leaves of another were inoculated from a 6-weeks-old corn-meal flask

culture (strain 33) and covered with bell jars. An uninoculated check tree was covered in the same way. The trees were sprinkled twice between June 15 and 20, and the bell jars were removed on the latter date, at which time definite infection was noted on the first inoculated tree, but none was on the second or on the check tree. An accident to these trees prevented further observations.

EXPERIMENT NO. 11 (Dec. 18, 1912).—The partly mature leaves of a seedling pecan were inoculated from an 8-weeks-old corn-meal-agar culture (strain 113). This and one check tree were left under a bell jar for three days, when tiny reddish brown specks could be recognized over the inoculated areas. After bleaching and staining, these leaves were examined for the mode of entrance of the fungus into the leaf. Many cases were found in which the mycelial threads had passed through the openings in the stomata. In all probability this mode of infection occurs in the field from the germination of the spores, but this point has not been proved by artificial infection, on account of the lack of distinct conidial formation in culture.

#### CULTURAL STUDIES

##### THERMAL TESTS

Several series of corn-meal-agar slant cultures were grown for two to three weeks in constant-temperature incubators ranging from 1° to 40° C. No growth took place below 5° or above 35°. After two to three weeks' incubation growth at 8° had barely started, while the rate gradually increased up to 30° (86° F.), this giving the highest rate for the temperatures tested. Growth at 32° was about the same as at 14°. Cultures incubated two to three weeks at 36° and 40° gave no signs of life when subsequently held at room temperature, while those incubated at 2° and 4° made a perfectly normal growth when placed under favorable conditions.

##### CULTURAL CHARACTERS

The cultural characters of the fungus as grown upon several of the more common media are briefly described below. No distinct development of conidia has been observed in cultures of the fungus.

Beef-Agar Slant Tubes.—The colonies are convex, approximately raw umber in color, glistening and smooth at first, but later becoming wrinkled and finally attaining a diameter of 10 to 12 mm. Aerial mycelium where present has been very sparse. The submerged mycelium consists of a pale-olive, tangled mass of hyphæ with many swollen and contorted cells.

Beef Broth.—The entirely submerged and dirty-whitish colonies consist of a rounded filmy mass of threadlike mycelium with but few swollen cells. Some of the hyphæ are beaded in appearance.

Corn-Meal-Agar Slant Tubes (Pl. XXXVII, fig. K).—The submerged growth which is usually the most prominent part is seal brown to black, while the somewhat cottony aerial mycelium is pinkish. After an incubation of one to two weeks a distinct violet tinge is assumed by the whole agar plug, and the combination of pigment and gelatinous medium gives an opalescent appearance to the whole. Colonies often reach a diameter of 15 mm. Except for the rather scant aerial mycelium, the growth is entirely below the surface of the medium where the hyphæ consist of more or less distorted, dark olive-brown cells.

Corn-Meal Flask Cultures.—The colonies are cottony to plushlike in surface appearance, with a wide variation of color comprising white to pale pink in the cottony parts and shades of raw sienna, burnt umber, and Venetian red elsewhere. A diameter

of 50 or more mm. is often attained by individual colonies after a growth of several weeks.

**Filter Paper.**—Growth on filter paper moistened with sterile distilled water caused the formation of dark reddish brown circular spots very similar in appearance to those formed on the leaf, while for a radius of 10 to 12 mm. around the spot the paper took on a pinkish cast. An extremely scant white to pinkish aerial mycelium was often developed.

**Oxalic-Acid-Agar Slant Tubes.**—Colonies are more or less convex, becoming wrinkled with age. The rather scant aerial growth is white to pale pink, while the submerged mycelium is seal brown to black and made up of densely anastomosing and variously contorted hyphae. The colonies are rarely over 10 mm. in diameter. After several weeks' growth the medium loses its pink color and assumes the shade of ordinary beef agar.

**Potato Cylinders.**—The colonies are very convex, with white to pinkish aerial mycelium and olive-gray surface growth which becomes much wrinkled with age. A diameter beyond 8 to 10 mm. is rarely attained. The potato cylinder assumes a dark-gray cast for several millimeters beyond the outermost fungous growth, due evidently to enzym action.

**Prune-Agar Slant Tubes.**—The colonies are little or not at all raised above the surface of the agar, with a fine, velvety, Indian red aerial growth. In the older and drier parts of the culture a scant white to pinkish aerial mycelium develops. A diameter of about 15 mm. is usually attained.

**Synthetic-Agar Slant Tubes (Pl. XXXVII, Fig. J).**—The colonies are extremely convex with a light to dark olive-green velvety surface growth. Numerous guttate drops of liquid are scattered over the surface during the earlier stages. A dark-brown to black, leathery pseudoparenchyma is developed beneath the surface, and with age the whole colony becomes considerably wrinkled. Growth continues until the agar has almost completely dried down, so that the whole slant surface of the medium is often eventually covered by the fungus.

#### MORPHOLOGY AND TAXONOMY

A comparison of the characters of this fungus with the description of a *Clasterosporium* published by Heald and Wolf and an examination of their type material deposited in the pathological herbarium of the Bureau of Plant Industry have shown that the two are undoubtedly the same species. Their description is as follows:

*Clasterosporium diffusum*.—Maculis indefinite marginatis, amphigenis; irregularibus, aequaliter brunneis, 5–10 mm. diam.; hyphis effusis prostratis, saepe laxe gregariis atque erectis; conidiis curvulis, clavatis, pluriseptatis, brunneis,  $45-135 \times 4-5 \mu$ .

On *Hicoria pecan* (Marsh) Britton. Victoria, 2536; Gonzales, 2695 (type); Yoakum 2770, Hallettsville, 2783.

This fungus produces circular or irregular, indefinite margined, brown spots, which are uniformly brown on both surfaces of the leaflets. Dark-brown hyphae run throughout the dead tissue or creep over either surface of the affected area, or are sometimes aggregated to produce clusters of erect conidiophores.<sup>1</sup>

After a careful study of this fungus from both the humid and semi-arid parts of the pecan belt it has seemed to the writer to conform more nearly with the *Cercospora* than with *Clasterosporium* characters.

Typically, the latter is saprophytic and possesses a prostrate or creeping mycelium with sporophores either short or differing but little from the

<sup>1</sup> Heald, F. D., and Wolf, F. A. New species of Texas fungi. *Mycologia*, v. 3, no. 1, p. 21, 1911.

conidia. The latter are borne singly, rarely in clusters, and are largely straight, with rounded ends.

The *Cercosporas*, on the other hand, are mostly parasitic, and form leaf spots. The sporophores are developed in thick bundles, either through the stomata and from mycelium within the leaf tissues which often takes the form of a stroma beneath the stomatal opening or by sporophores breaking through the epidermis irregularly. The conidia are longish-cylindrical or spindle-shaped, occasionally somewhat club-shaped, straight or bent, and often with a long drawn-out point.

As observed in the humid sections, the typical forms of this fungus had the densely clustered sporophores which, occurring mostly on the upper leaf surface, have arisen from a stroma breaking through the epidermis rather than through the stomatal openings. (Fig. 3.) The mycelium is largely within the leaf tissue and is intercellular, but is also found creeping over the leaf surface and giving rise here and there to single conidiophores. In the semiarid sections the latter type of spore

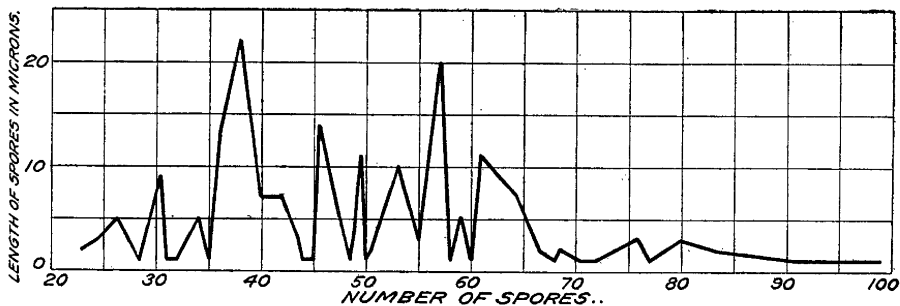


FIG. 4.—Diagram showing measurements in length of 200 conidia.

formation appears to be the more frequent. The conidia are long, usually somewhat club-shaped, and with the apical end the more pointed.

It will be seen that the fungus possesses some characters of both genera. However, since under conditions favorable to fungus growth the *Cercospora* characters greatly predominate, it has seemed best to place it in this genus. Of course parasitism or nonparasitism should scarcely be given a generic value, but this point at least adds further weight to the present decision. Furthermore, since a *Cercospora diffusa* has been previously described by Ellis and Everhart<sup>1</sup> as occurring upon leaves of *Physalis lanceolata*, it becomes necessary to change also the specific name of this pecan fungus. The emended description of the fungus is given below.

***Cercospora fusca*, emend. sp.**

Syn. *Clasterosporium diffusum* Heald and Wolf.

Leaf spots up to 10 or 15 mm. in diameter, at first somewhat angular and bounded by the veins, becoming roundish to irregular and with more indefinite margin, dark reddish brown on both leaf surfaces.

<sup>1</sup> Ellis, J. B., and Everhart, B. M. Additions to *Ramularia* and *Cercospora*. Jour. of Mycol., v. 4, no. 1, p. 3, 1888.

Mycelium dark brown and septate, intercellular, sometimes also creeping over the leaf surfaces.

Conidiophores mostly epiphyllous, short and erect, typically in dense, tawny clusters from stromata developed beneath the epidermis and later bursting through, also arising singly from the prostrate surface mycelium.

Conidia pale olive brown, highly variable in size, 30 to 100 $\mu$  or more by 3 to 6 $\mu$  (see figs. 4 and 5), usually curved, typically subclavate, multicellular, septa less frequent toward the more pointed apical end.

Habitat.—Living leaves of *Carya illinoensis*, Southern States. Also possibly occurring on other species of *Carya*. Diseased nuts or leaves seen by the writer at Orangeburg, Sumter, and Charleston, S. C.; Americus, Albany, DeWitt, Hardaway, Baconton, Thomasville, Cairo, Bainbridge, and Valdosta, Ga.; Tallahassee, Newport, Monticello, Glen St. Mary, St. Augustine, Palatka, Gainesville, Ocala, and Belleview, Fla.; New Orleans, La.; and at Waring and San Antonio, Tex. Reported also by Heald and Wolf<sup>1</sup> from Victoria, Gonzales, Yoakum, and Halle'sville, Tex.

### PECAN ANTHRACNOSE

[Caused by *Glomerella cingulata* (Stonem.) S. and v. S.]

#### HISTORY AND DISTRIBUTION

Pecan anthracnose, variously known among pecan growers as "leaf-blotch" and "rust," was first noted by the writer during the summer and fall of 1910, at which time single-spore strains of the causal fungus were obtained from perithecia matured on the leaves in a damp chamber. Studies of these cultures were carried out during the following winter, and a preliminary description of the fungus later appeared under the name of *Mycosphaerella convexula*.<sup>2</sup>

Further cultural studies of the fungus brought out changes in its morphology sufficient to throw it out of the genus *Mycosphaerella*, and these, together with cross-inoculation experiments upon the apple, indicated its close affinity to the apple bitter-rot caused by *Glomerella cingulata*.<sup>3</sup> No other published information concerning this disease has come to the notice of the writer.

Pecan anthracnose seems to be well distributed throughout the eastern part of the pecan-growing territory, but it has thus far usually occurred only to a limited extent at any one place. Diseased leaves or nuts have been collected by the writer at Orangeburg, Sumter, Summerton, Charleston, and Aiken, S. C.; at numerous places in southern Georgia and

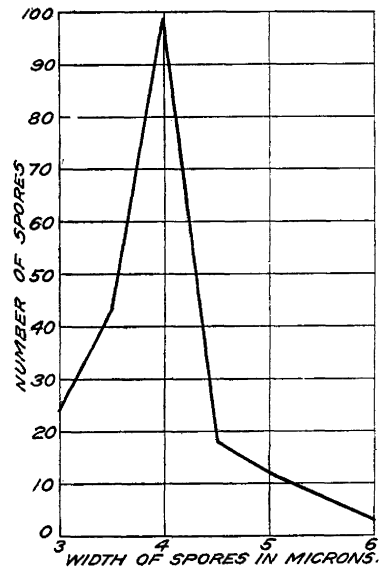


FIG. 5.—Diagram showing measurements in width of 200 conidia.

<sup>1</sup> Heald, F. D., and Wolf, F. A. Loc. cit.

<sup>2</sup> Rand, F. V. A pecan leaf-blotch. *Phytopathology*, v. 1, no. 4, p. 133-138, 3 fig., 1911.

<sup>3</sup> Rand, F. V. Further studies on the pecan "rust." *Science*, n. s., v. 35, no. 913, p. 1004, 1912.

northern Florida, including Albany, Dewitt, Baconton, Thomasville, Cairo, and Bainbridge, Ga., and Tallahassee, Newport, Monticello, Jacksonville, St. Augustine, and Belleview, Fla.; and at San Saba, Tex.

#### SYMPTOMS OF THE DISEASE

The disease has been found to occur on both leaves and nuts of the pecan. On the leaves it appears in the form of irregular, reddish to grayish brown blotches varying greatly in size and eventually often covering the whole leaf. (Pl. XXXVII, fig. *B*.) The color of the affected areas is the same on both surfaces. Under conditions of moderate humidity, spores of the *Gloeosporium* type are developed singly, but with favorable temperature and moisture the acervuli with exuding pink spore masses and the black perithecia appear rather thickly scattered over the diseased blotches. When the greater part of the leaf blade becomes involved, it usually falls to the ground, and it is here, under natural conditions, that the acervuli and perithecia are developed.

The blotches on the nuts are also irregular in outline, but are nearly or quite black and often slightly sunken below the surrounding healthy tissue. (Pl. XXXVII, fig. *F*.) The perithecia and the densely gregarious acervuli are formed under the same conditions as on the leaves, but the perithecia have been found on the nuts with much less frequency. A serious dropping of the partially grown nuts sometimes occurs from this cause.

A watery condition of the kernel is frequently found in connection with the anthracnose blotches. It is doubtful, however, whether it has anything to do with this disease, for the same condition prevails both with and without external signs of injury, while both cultural methods and microscopical study have thus far failed to locate any microorganisms in the watery kernels.

#### MYCOLOGICAL AND PATHOLOGICAL STUDIES

##### ISOLATION OF THE FUNGUS

No mature perithecia have as yet been found on fresh leaves or nuts, but at various times during the last three seasons ripe asci have been readily developed on affected leaves after an incubation of one or two weeks in a damp chamber. The original strains of the fungus were obtained in this way from nursery-tree leaves collected in the fall of 1910 at Baconton, Ga., and were each started from a single, apparently 2-celled ascospore the germination of which was closely followed under the microscope to preclude the possibility of contamination. On several different culture media the colonies at once developed perithecia suggesting the genus *Mycosphaerella*, and the great majority of the slightly curved ascospores were apparently 2-celled, though a few showed no signs of a cross-septum.

After carrying in culture for about two months, a few spores were noted which were 1-celled, oblong, and slightly smaller than the typical ascospores. These conidia as first noted were borne hyphomycetously, but later were found developing from dense groups of modified fungous cells like an acervulus and in size and shape resembling a typical *Gloeosporium*. For some time it was thought that this was a contamination, though no possibility of such an occurrence could be found. However, in order to determine this point with certainty, single-spore cultures were started from the 2-celled ascospores and also from the conidia, each individual spore being isolated and its germination carefully followed microscopically. The resulting two series of cultures were similar macroscopically, and both soon developed typical perithecia and ascospores, and also the *Gloeosporium* conidia. This procedure was repeated 30 or 40 times with a like result in all cases. In several instances the germinating ascospores had within 24 to 48 hours developed mycelial threads which were cutting off conidia in considerable numbers, and in these cases the hyphal connection could frequently be traced from the parent ascospore to the conidium.

However, it was noted after eight or nine months' growth in culture that the 2-celled ascospores were becoming fewer in proportion to the 1-celled, and this tendency has continued until now, after more than two years in culture, the majority of the ascospores are 1-celled, though still of the original form and size.

The possibility suggested itself that perhaps many of the apparent septa were in reality merely a denser layer of cytoplasm across the center of the single cell and that after many generations of growth in culture this cytoplasm had for some reason become more homogeneous. Whatever the explanation, the fact remains that in these original strains a change has taken place from a majority production of apparently 2-celled to that of 1-celled ascospores and that the production of acervuli has become quite as abundant as that of the perithecia. It should be added, however, that many of the ascospores possessed an undoubted septum, as clearly brought out by staining.

During the last two years 10 or 12 other strains of the fungus have been obtained from both conidia and ascospores developed on naturally infected leaves and nuts. In these cases most of the ascospores have been unicellular, though a few have been found with a cross partition clearly brought out by staining.

#### INOCULATIONS

Several series of inoculation tests have been carried out on the leaves, but on account of the exigencies of field travel and the difficulty of obtaining suitable material and conditions only two sets of infection experiments have been tried on the nuts.



From the similarity of this fungus to the *Glomerella* rot of apples and from the omnivorous character of the latter species, as brought out in a paper read by Shear<sup>1</sup> at the 1911 meeting of the American Association for the Advancement of Science, it was decided to make several cross-inoculation tests on the apple. The results of inoculation tests on leaves and nuts and of the cross-inoculation work on the apple are given in the following pages.

#### LEAVES

EXPERIMENT NO. 1 (Feb. 16, 1911).—A distilled water suspension of ascospores from a month-old corn-meal flask culture (strain 17, Baconton, Ga., 1910) was sprayed upon the lower surfaces of six potted pecan seedlings. Three of the seedlings were under bell jars for four days, while the remaining three were left uncovered throughout the experiment. Observations at the end of a week showed no signs of infection, but at four weeks numerous small discolored areas had developed on the foliage of the first three trees and on that of all but one of the others. The three check trees which had been sprayed with distilled water and left under bell jars for four days were sound. No further development of the disease was apparent for some time, but during the latter part of June large, dull reddish brown areas were noted on the leaves of the first three inoculated trees and on one of those which had not been covered with a bell jar. Specimens of these diseased leaves were at once collected, and a microscopical examination showed the development of an occasional *Gloeosporium* conidium. The leaves were then placed in a damp chamber, and after several days numerous acervuli had developed and were exuding the typical pink spore masses in abundance. Reisolations of the fungus were made from these acervuli.

EXPERIMENT NO. 2 (Mar. 15, 1912).—Conidia (strain 17) from 2-weeks-old corn-meal-agar cultures were mixed with sterile distilled water and sprayed upon the leaves of four potted seedling trees, which were then left under bell jars for five days. Two check trees were sprayed with sterile distilled water alone, one being left under a bell jar for five days and the other uncovered. After four weeks it was noted that discolored areas similar to those noted in inoculation experiment No. 1 had suddenly developed, but observation at ten weeks showed no further progress of the disease. The last of May, however, when the leaves were getting well matured, the large, dead, brownish areas were fairly numerous on the leaves of three out of the four inoculated trees. The check trees which had been kept on the same bench but somewhat removed from the infected trees were entirely normal. Specimens of the infected leaves were placed in a damp chamber, where in a few days the *Gloeosporium* acervuli were formed.

EXPERIMENT NO. 3 (Apr. 15, 1912).—Conidial masses from a young corn-meal-agar culture (strain 17) were smeared upon both surfaces of the moistened leaflets of two potted seedlings, one of which was left under a bell jar for several days. Two check trees were similarly treated, but not inoculated. No discoloration of the leaves followed for several weeks, but on May 20 several dead, brownish areas were noted on the leaves of the inoculated tree which had been under a bell jar. These leaves were placed in a damp chamber and in a week had formed numerous acervuli with the typical pink spore masses.

EXPERIMENT NO. 4 (May 1, 1912).—Two of the younger leaves from a Sovereign pecan were placed in a damp chamber and sprayed with a sterile distilled-water suspension of conidia from a 2-months-old corn-meal-agar culture (strain 17). The surface of some of the leaflets was slightly abraded with a needle before inoculation. At the end of two weeks large dull-brown areas had developed on most of the leaflets, both

<sup>1</sup> Shear, C. L. Variation in *Glomerella*. (Abstract.) *Science*, n. s., v. 35, no. 891, p. 152, 1912.

where abraded and where the surface was left intact. The largest of these irregular spots covered as much as half the surface of the leaflets, and numerous perithecia were forming, though only a few were mature at this time. When 3 weeks old the spots had increased in area so as to involve most of the tissue, and most of the perithecia were mature, bearing the typical curved ascospores in abundance. No acervuli or scattered conidia were noted.

EXPERIMENT No. 5 (May 29, 1912).—Twenty young seedling pecan leaves were placed in damp chambers and lightly sprayed with a distilled water suspension of conidia and ascospores from a 5-weeks-old corn-meal-agar culture (strain 17). On the third day small brownish areas had developed here and there over the leaf surfaces. On the eighth day these had nearly covered the leaves, and numerous perithecia, together with an occasional acervulus, had developed in the dead tissue. (Pl. XXXVII, fig. B.) These fruiting bodies occurred in greater abundance on the lower side of the leaves, but frequently on both upper and lower surfaces. The incipient perithecia and acervuli developed beneath the epidermis, but later burst through and became partly superficial.

EXPERIMENT No. 6 (Oct. 22, 1912).—Six vigorous but mature leafy pecan shoots were sprayed with a sterile distilled-water suspension of conidia from strain 123 obtained from diseased nuts, and a similar number with strain 150 obtained from a naturally infected apple. The shoots were cut under water and the lower ends placed in bottles of water under slightly ventilated bell jars. Nine check shoots were treated in the same way, except that they were not inoculated.

At three days many of the inoculated leaves in both sets had begun to show the dead, brownish areas characteristic of the disease. After seven days these areas had in some cases involved nearly the whole leaf, with the development of acervuli in moderate numbers. The check leaves were still green and healthy.

EXPERIMENT No. 7 (Mar. 25, 1913).—Distilled-water suspensions of conidia from one apple strain and three pecan strains of the fungus were sprayed over young seedling pecan leaves in damp chambers. After three days sample leaves from each set were collected and prepared for microscopical examination. A small percentage of the conidia, varying somewhat with the different strains, had sent out germ tubes. Some of the short hyphæ were terminated by appressoria. In one or two cases the germ tube was traced into the opening of a stoma. This method of infection agrees with that described by Shear<sup>1</sup> for *Gloeosporium* on a wide variety of hosts.

After five days several of the leaflets in each set exhibited typical infection areas up to 30 mm. in diameter. However, on account of a field trip, no further observations were made on this experiment.

#### NUTS

EXPERIMENT No. 1 (Oct. 22, 1912).—These inoculations were from strain 123, obtained in October, 1912, from blackened nut shucks sent in from Thomasville, Ga., by Mr. C. A. Reed. Terminal shoots bearing healthy green pecans were kindly furnished by Mr. J. B. Johnson, of Manassas, Va. These shoots were cut under water to prevent clogging of the vascular system, placed with the cut ends in bottles of water, and sprayed with a distilled-water suspension of the conidia from this strain. All were then covered with bell jars ventilated at the base to prevent a too great stagnation of the air, but at the same time to furnish sufficient humidity to insure germination of the spores. The check shoots were treated in the same way, except that they were sprayed with distilled water alone.

Group A consisted of 7 shoots bearing 9 nuts, the hulls of which were punctured with a sterile needle and sprayed with sterile distilled water. Group B consisted of 2

<sup>1</sup> Shear, C. L., and Wood, Anna K. Studies of fungous parasites belonging to the genus *Glomerella*. U. S. Dept. Agr., Bur. Plant Indus. Bul. 252, 110 p., 18 pl., 4 fig., 1913.

shoots treated in the same way but unpunctured. Groups A and B were held as checks. Group C consisted of 8 shoots bearing 10 nuts the hulls of which were punctured with a sterile needle and sprayed with a sterile distilled-water suspension of the conidia. Group D consisted of 6 shoots bearing 8 pecans which were inoculated like group C, except that the hulls were not punctured. Group E consisted of 10 nuts removed from the shoots, their hulls punctured, and inoculated with a suspension of conidia as in groups C and D, and then placed in damp chambers.

At the end of three days distinct infection had occurred on all the nuts with puncture inoculations, the tissue of the hulls being blackened for a radius of 2 to 5 mm. around the needle punctures. The first checks had the tissue blackened for a radius of about 0.5 mm. around the needle punctures, while the nonpunctured check and inoculated nuts at this time showed no evidence of infection. Many of the leaves on the inoculated shoots were developing small, irregular brownish areas, while the uninoculated leaves were all green and healthy.

After nine days groups A and B appeared as on the third day. The very narrow margin of blackened tissue in the punctured checks was due merely to the mechanical injury to immediately surrounding cells, and no further injury occurred throughout the experiment. (Pl. XXXIII, fig. 1, A.) All the pecans in group C (Pl. XXXIII, fig. 1, C) were blackened over half to the whole of their surface, and acervuli were beginning to develop over the dead parts, with an occasional exudation of the pink spore masses. In group D half of the eight nuts had blackened, and acervuli had begun to develop, but the others gave no evidence of infection. (Pl. XXXIII, fig. 1, B.) In group E all the nuts were blackened, and very numerous acervuli with exuding spore masses had developed. Reisolations of this fungus were made as strain 144. Plate XXXIII, figure 2, shows three of the inoculated nuts after further development of acervuli.

#### APPLES

EXPERIMENT NO. 1 (Dec. 30, 1911).—Five sound Jonathan apples direct from cold storage were placed in damp chambers and inoculated by needle punctures, two of them with conidia and three with ascospores inserted directly into the punctures. Three sound apples were punctured with sterile needles and also placed in damp chambers. All were kept overnight at 35° C., and subsequently throughout the experiment at laboratory temperatures. Examination after seven days showed a decay very similar in appearance to bitter-rot around all the inoculation punctures. The check apples were perfectly sound. These cultures were kept for 10 days, with a gradual increase in the size of the decayed areas and formation of incipient fruiting bodies but no spore production.

EXPERIMENT NO. 2 (Mar. 5, 1912).—Twelve Yellow Newtown apples were similarly inoculated and placed in damp chambers, one half being inoculated with conidia and the other half with ascospores from an 8-weeks-old corn-meal-agar culture (strain 17). Three apples from each set were held at 28° to 30° C. and three from each set at laboratory temperature. Six apples punctured with sterile needles and placed in damp chambers were held as checks, one half at 28° to 30° and the other half at laboratory temperature. At the end of a week the cultures were examined, and all the inoculated apples had developed a decay apparently identical with bitter-rot, but the brownish and somewhat sunken spots had increased in size much more rapidly at the higher temperature. Two weeks later perithecia were forming and the conidia were developing in considerable numbers, but not in such amount as to give the pink spore masses characteristic of bitter-rot. The check apples at both temperatures remained sound to the end of the experiment.

EXPERIMENT NO. 3 (Nov. 30, 1912).—Sound Jonathan and Yellow Newtown apples direct from cold storage were inoculated with three strains of *Glomerella* obtained from the pecan and with one strain obtained from the apple. The cultures used

were all young corn-meal-agar-slant tubes of the same age and bearing the *Gloeosporium* stage in abundance. Inoculations were by needle puncture in damp chambers, and, with the exception of group A, all were held at 28° to 30° C. for 48 hours; after this they were kept at laboratory temperature. Group A was held at laboratory temperature throughout.

Group A consisted of three Jonathan apples which were inoculated with strain 17, isolated from diseased pecan leaves collected at Baconton, Ga., in the fall of 1910.

Group B consisted of three Jonathan and four Yellow Newtown apples inoculated with strain 123, isolated in October, 1912, from diseased nuts from Thomasville, Ga.

Group C consisted of four Yellow Newtown apples inoculated with strain 125 similarly obtained from diseased nuts collected at Sumter, S. C., in October, 1912.

Group D consisted of three Yellow Newtown apples inoculated with strain 150, obtained in October, 1912, from an apple naturally affected with bitter-rot.

Group E consisted of six Jonathan and four Yellow Newtown apples treated similarly but not inoculated.

On the fourth day typical bitter-rot areas had developed in all the inoculated cultures. In group A the spots were 1 to 3 mm., while in B to D they were 3 to 20 mm. in diameter. It should be stated that the progress of the tissue decay was somewhat more rapid with strains 125 and 123 than with 150, obtained from the apple itself. In all cases the pale pinkish white mycelium could be seen protruding in tufts from the needle punctures, and the dark-colored fruiting bodies were developing. There were conidia evident at this time. The check apples remained sound. (Pl. XXXIV, fig. A.)

On the eleventh day the spots had increased considerably in size, many of them being 15 to 20 mm. in diameter and becoming confluent. (Pl. XXXIV.) Acervuli extruding the pink spore masses occurred in dense aggregations over the infected tissues, being considerably more abundant, however, in strains 123 and 150 than in the other two. No perithecia had developed as yet, and even after six weeks none had appeared except on the apples inoculated with strain 123.

EXPERIMENT NO. 4 (Mar. 21, 1913).—Sound Yellow Newtown apples direct from cold storage were inoculated as in experiment 3 with two strains of the fungus obtained from diseased nuts, one each from the pecan leaf and the apple, and one originally from the nut but reisolated from an artificially inoculated apple.

On the fourth day bitter-rot areas had developed about the needle punctures in the case of every strain tested, while the check apples remained perfectly sound. (Pl. XXXV.) The decaying spots rapidly increased in size, and after eight days the formation of acervuli had begun.

From these inoculation tests it would appear that this fungus is parasitic on the leaves of the pecan, though usually not actively injurious until a certain stage of maturity of the leaves is reached, together with favorable conditions of temperature and humidity. Field observations also bear out this point.

The limited inoculation work with the nuts, taken alone, would hardly justify very definite conclusions, but as far as they go the experience with leaf inoculations is duplicated. No artificial infection tests have been made upon very young nut hulls, but from the field observations of the last two seasons no evidence of injury during the early part of the season has been obtained. The disease has come to notice only from mid-season on until fall. These observations are in line with the seasonal distribution of bitter-rot as it occurs on the apple.

The cross-inoculations upon the apple, carried simultaneously with infections by the apple bitter-rot fungus, show that the pecan fungus from both leaves and nuts is at least physiologically similar to the *Glomerella* of the apple. The morphological characters will be discussed later.

#### CULTURAL STUDIES

##### THERMAL TESTS

Several series of corn-meal-agar cultures were grown for two to three weeks at temperatures ranging from 1° to 35° C. As a result of these studies it was found that no growth would take place below 6°, either with freshly inoculated cultures or with those in which growth had already started before incubation. At 7° to 8° the growth was extremely slow, but gradually increased with rise of temperature until the maximum for the strains tested was reached at about 30°.

##### CULTURAL CHARACTERS

Since the fall of 1910 various strains of the fungus have been grown on the common culture media, and their appearance under different conditions is briefly given as follows:

**Beef-Agar Slant Tubes.**—The colony first appears as a colorless, roundish, submerged mycelial mass which at ordinary temperatures generally covers the slant in five to seven days, while one to several groups of acervuli or black perithecia have in the meantime usually begun to form. The growth is at first entirely submerged and the surface of the slant presents a smooth glistening appearance. However, after something like two weeks a small amount of whitish aerial mycelium makes its appearance toward the upper edge of the slant. In old cultures this subicle may sparsely cover the whole surface, while the submerged parts become very dark colored.

**Corn-Meal Flasks.**—Growth becomes visible in two to three days as a roundish colony several millimeters in diameter, with sparse, white to pinkish, cottony aerial mycelium in which are usually scattered a considerable number of dark olive-brown dots. These dots are found to consist of numerous interwoven hyphæ with swollen and contorted cells in process of uniting to form a pseudoparenchyma. These dark masses later develop either into acervuli or perithecia.

**Corn-Meal-Agar Slant Tubes.**—The white to colorless growth is at first submerged or at the surface. After several days acervuli or perithecia usually begin to form and a scant whitish aerial mycelium may appear at the edges of the slant. The pink spore masses are often developed without the formation of a dark-colored stroma, while in other cases this stroma may be the most conspicuous part of the acervulus. The perithecia are developed within black carbonaceous masses of mycelium which may or may not be submerged in the medium. In old cultures parts of the submerged growth often become olive green to almost black.

**Cooked-Potato Cylinders.**—Growth first becomes evident through a light-brown discoloration of the tissue immediately around the point of inoculation, and usually a whitish aerial tuft of mycelium appears within 24 hours at the center of the discolored area. This breaking down of the tissue progresses rapidly so that after several days the whole cylinder becomes involved. The white to pinkish cottony subicle develops somewhat more slowly, but eventually covers the cylinder and bears the embedded acervuli or perithecia.

## PEDIGREED CULTURES

Starting with a single ascospore and a single conidium from the same strain of the fungus, two series of corn-meal-agar cultures were carried for five generations. Each generation was grown for three weeks before transfers were made for the next succeeding generation, and conditions of temperature and medium were made as uniform as possible throughout the 15 weeks of the test. Observations in every case were taken at three weeks. In the first strain ascospores were always used in making the transfers, while conidia alone were transferred in the second strain.

**Ascospore Strain.**—Generation 1 had numerous black, carbonaceous, perithecial groups and no acervuli, though a moderate number of conidia were developed hyphomycetously.

In generation 2 the perithecia and acervuli occurred in about equal numbers. In many cases the black perithecial clusters were surrounded with acervuli which were exuding pink masses of spores.

Generation 3 exhibited dense black masses of perithecia near the base of the slants and a considerable number of acervuli which were mostly toward the upper part.

Generations 4 and 5 were similar to the last, except that the two forms were more uniformly scattered over the surface of the cultures.

**Conidial Strain.**—Generation 1 had numerous acervuli with exuding pink spore masses, but no perithecia.

Generations 2 and 3 had numerous perithecial groups and acervuli well scattered over the cultures, with neither form greatly in predominance.

Generation 4 had numerous pink spore masses along the streak, and perithecial clusters in moderate numbers near the base of the slant.

Generation 5 had both forms in about equal numbers and well scattered over the surface of the cultures.

Further cultural studies carried out in the same way as the one described above have given essentially the same results—namely, that a strain producing both spore forms will continue to produce both ascospores and conidia even though one form alone is used in reproduction. Variations have occurred from time to time, but these have occurred irregularly and without continuance. Strains of the fungus from single ascospores have sooner or later always given rise to both ascigerous and conidial forms. However, some conidial strains have been obtained from the host which, after two years in culture, still produce only the conidial form. It would thus appear that there are conidial strains of the fungus which have lost the power of developing the perfect stage or which at least have not met with the proper inciting conditions.

## MORPHOLOGY AND TAXONOMY

The perfect stage has been noted less frequently than the conidial stage, but nevertheless the perithecia have been occasionally found on both leaves and nuts. The first evidence of perithecial formation is seen in a plexus of pseudoparenchyma tissue made up of more or less isodiametric fungous cells developed in the decaying tissues beneath the epidermis. This finally develops into the mature perithecium which ruptures the

epidermis and becomes partially superficial. The mature fruiting body is nearly spherical, but is papillate and occasionally short beaked. From several hundred measurements it has been found to vary from 80 to 250 $\mu$  in the longest diameter, with the majority lying between 150 and

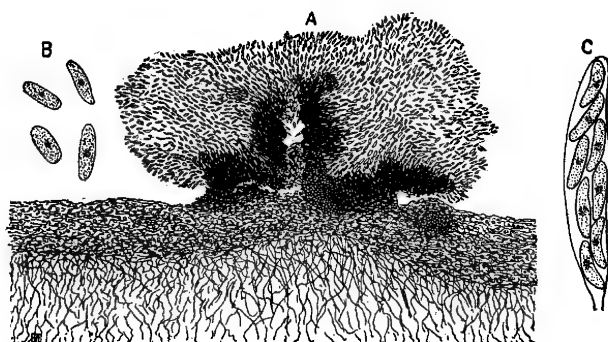


FIG. 6.—The anthracnose fungus upon corn-meal agar. A, Acervulus,  $\times 84$ ; B, conidia,  $\times 400$ ; C, ascus,  $\times 400$ .

200 $\mu$ . The perithecia are black and carbonaceous, and in culture several are usually developed within a single carbonaceous stroma.

The 8-spored asci vary considerably in size and shape, but are usually cylindrical-clavate. (Fig. 6.) The

extreme measurements found were 45 to 80 by 9 to 12.5 $\mu$ , the majority lying within the limits of 55 to 80 by 10 to 11 $\mu$ .

The ascospores are unicellular (rarely with a cross partition), oblong, slightly tapering toward both ends, and usually curved. (Fig. 6.) The extreme measurements found were 12.5 to 29 by 3.5 to 6 $\mu$ , the majority lying about midway between the two extremes as shown in the accompanying graph (fig. 7) drawn from measurements of 150 spores of a single strain taken at random and all developed in corn-meal-agar culture. Measurements of other strains both from culture and from the host have come within these limits.

The acervuli have been of much more common occurrence on the host. (Fig. 6.) In their early stages they are scarcely to be distinguished from the perithecia, but the production of the characteristic pink spore masses soon differentiates them even macroscopically from the perfect stage. The production of setae has been found of frequent though by no means of general occurrence, and to vary even within a single strain.

The conidia are ovate to oblong, with blunt, rounded ends (fig. 6) (occasionally somewhat dumbbell-shaped). Both on the host and in culture they have been found to develop hyphomycetously, as well as in acervuli. The measurements taken from several strains on the host



FIG. 7.—Diagram showing ascospore measurements of the anthracnose fungus. A, Length of 150 ascospores; B, width of 150 ascospores.

and in culture ranged within the limits of 11 to 22 by 3.8 to 7.6 $\mu$ . The accompanying graph (fig. 8) shows the measurements of 150 conidia taken from the same strain and under the same conditions as those noted above for the ascospores. The conidia have frequently been found to develop appressoria as described by various authors for the apple bitter-rot fungus.

From the general pathology and temperature relations, the cross-inoculation and cultural studies, and finally from the morphology of the pecan fungus there can be no doubt of its specific connection with *Glomerella cingulata* (Stonem.) S. and v. S., the fungus causing bitter-rot of apple, ripe-rot of grape, and anthracnoses of a wide range of hosts.

In several instances *Glomerella perithecia* have developed upon pecan leaves scattered among the densely gregarious pycnidia of a fungus which has since proved to be *Phyllosticta convexula* Bubák.<sup>1</sup> The spores of the latter are almost bacillar in size, measuring 1.5 to 2 by 1 $\mu$ , while in many cases only a few pycnidia upon a leaf matured in damp chamber, so that morphologically most of these fruiting bodies were similar to the immature perithecia of *Glomerella*.

Furthermore, an examination of the fruiting bodies from type specimens of *Sphaerella convexula* (Schwein.) von Thüm.<sup>2</sup> (*Sphaeria convexula* Schwein.<sup>3</sup>) shows them to be morphologically similar to those of *Phyllosticta convexula*. The original brief diagnosis of *Sphaerella convexula* was from immature material and without description of asci or ascospores. Similar material has been collected by the writer at various points in South Carolina, Georgia, and Florida, including one of the type localities of the species.

*Glomerella perithecia* have been developed in a damp chamber, not only upon disinfected pecan leaves exhibiting the typical anthracnose blotches and among the pycnidia of *Phyllosticta convexula*, but also frequently upon leaves apparently healthy in every respect, showing the wide distribution of the former fungus and its ability to hibernate on the living host until the occurrence of conditions favorable to its further growth.

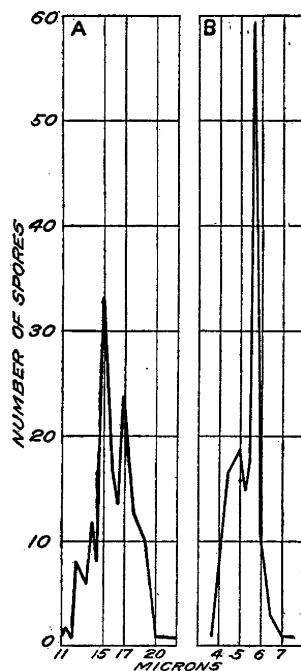


FIG. 8.—Diagram showing conidial measurements of the anthracnose fungus. A, Length of 150 conidia. B, width of 150 conidia.

<sup>1</sup> Bubák, Franz, Einige neue Pilze aus Nord America. Jour. Mycol., v. 12, no. 82, p. 52, 53, 1906.

<sup>2</sup> Saccardo, P. A. Sylloge Fungorum. v. 1, Patavium, 1882, p. 494.

<sup>3</sup> Schweinitz, L. D. von. Synopsis fungorum in America boreali media degentium. Trans. Amer. Phil. Soc. n. s., v. 4, p. 224, 1834.

Berkeley, M. J. Notices of North American fungi. Grevillea, v. 4, no. 32, p. 154, 1876.



From these facts it seems entirely possible, if not indeed probable, that the type fungus of Schweinitz and Von Thümen was in reality identical with *Phyllosticta convexula* Bubák and that the immature perithecia were those of the fungus at present known as *Glomerella cingulata*.

### KERNEL-SPOT

[Caused by *Coniothyrium caryogenum*, n. sp.]

### HISTORY AND DISTRIBUTION

Fortunately this disease has hitherto been of only occasional occurrence. In the fall of 1907 infected kernels were received by Mr. W. A. Orton, Pathologist in Charge, Cotton and Truck Disease and Sugar-Plant Investigations, Bureau of Plant Industry, from Minden, La., accompanied by the following statement:

The disease of the pecans is not confined to any one tree or variety. \* \* \* For six years they have contained the blight, growing worse each year, until I think that next year there will not be a single good one (nut) found among them. \* \* \* I have never heard of this disease from anyone else. All our trees are infected.

From these specimens Orton isolated a fungus with brown, septate, branched mycelium. No further studies were carried out to determine its parasitism or further cultural characters,<sup>1</sup> but examination of these specimens has shown them to have the symptoms associated with the kernel-spot.

No other definite reports of the kernel-spot prior to 1910 have come to notice, but during the last three years occasional specimens from a number of Southern States have been received by the Office of Fruit-Disease Investigations. Among these communications the only report of serious injury was from a point in southern Georgia, where in the fall of 1911 most of the nuts on a large seedling tree were rendered unfit for consumption. From this source were obtained the fungous cultures used in the present inoculation work. Since there were no nuts on this tree the following year, field studies as to time and manner of infection could not be carried further.<sup>2</sup>

### SYMPTOMS OF THE DISEASE

Externally there is no evidence of infection and it is only upon freeing the kernel from the shell that the diseased condition becomes apparent. (Pl. XXXVII, fig. E.) On the surface of the kernel the spots are dark brown to almost black and often slightly sunken. They are in general irregularly roundish with a fairly definite margin and several millimeters in diameter. Internally the diseased tissue extends in an approximate

<sup>1</sup> Orton, W. A. From unpublished notes.

<sup>2</sup> If a tree is badly infected, the nuts should be gathered and burned, in order to lessen the chances of further spread of the disease.

hemisphere beneath the dark-colored spot. The central part of this hemisphere is dry and pithy, slightly discolored, and surrounded by a definite dark-brown layer separating the diseased from the healthy parts. The tissues are slightly disorganized, but are not softened or entirely broken down. A bitter taste is imparted to the kernel. Microscopically, the fungus is found to enter the cells of the kernel, where the hyphæ become partially broken up into their constituent cells. Outside the dark-colored boundary layer the tissues of the kernel are seen to be slightly discolored, although no signs of the fungus itself are seen here. It seems probable that enzymes or toxins (or both) excreted by the fungus may diffuse out into the healthy cells of the host and by partial digestion prepare the way for the entrance and progress of the parasite.

#### MYCOLOGICAL AND PATHOLOGICAL STUDIES

##### ISOLATION OF THE FUNGUS

The affected pecan kernels received in the fall of 1911 from Thomasville, Ga., were washed for five minutes in a solution of bichlorid of mercury (1:500), and in distilled water. Small pieces of the diseased internal tissue were then cut out under sterile conditions and transferred to Petri dishes of melted beef agar. Yellowish bacterial colonies resulted from two of the transfers, but a constant fungous type developed from all the others. The bacteria and the fungus were isolated and carried in pure culture for the following cultural and inoculation studies.

##### INOCULATIONS

In all the inoculations the kernels were freed from the shells under semisterile conditions and placed upon sterile, moist filter paper in Petri dishes. Under these conditions the pycnosporos or mycelium from a pure culture were placed upon the kernels either with or without slight abrasion of the surface. The checks were treated in a similar manner but without inoculation.

EXPERIMENT NO. 1 (Jan. 15, 1912).—The kernels from several stratified nuts were placed in Petri dishes and inoculated by slight abrasion (1) with spores of the fungus (strain 99), and (2) with the yellow bacteria (strain 101), while the kernels in the third dish (3) were merely abraded with a sterile scalpel. After eight days typical symptoms of the kernel-spot had developed in the first culture. (Pl. XXXVII, fig. D.) The bacteria in the second culture had made a slight growth, causing an irregular softening of the superficial tissues, but without discoloration or other resemblance to the kernel-spot. The check cultures were entirely sound.

EXPERIMENT NO. 2 (Jan. 25, 1912).—Kernels of well-cured Stuart pecans were inoculated with the fungous spores, six kernels upon the uncut surface, and eight with a slight abrasion. Four kernels were held as checks. After 12 days typical spots had formed upon half of the first set and on all of the second set of kernels. Of the checks, two kernels were perfectly sound, the third exhibited a slight bacterial softening at one end, and the last was softened throughout by a growth of *Penicillium*

*glaucum*. In the last two cases the injury was similar in no particular to the kernel-spot.

EXPERIMENT NO. 3 (May 13, 1912).—Three Petri dishes containing four to eight kernels from cured pecans were inoculated by placing macerated pycnidia upon the uninjured surfaces. A fourth Petri dish was held as a check. After seven days it was noted that infection had taken place at every point of inoculation in the first two cultures. In the third, two kernels had become infected with the kernel-spot, but the remaining two were entirely softened by bacterial contamination. In the check Petri dish two kernels were sound and two were contaminated and softened throughout by *Botrytis cinerea*. In no case was the injury by contamination similar to the disease under investigation.

EXPERIMENT NO. 4 (Nov. 20, 1912).—Ten kernels of newly harvested pecans were inoculated with macerated pycnidia and without abrasion of the surface skin. A similar number of kernels were held as checks. After nine days, 8 out of the 10 inoculated kernels had developed the disease. The checks were sound, except for two or three kernels which had softened and yellowed throughout from bacterial contamination.

EXPERIMENT NO. 5 (Dec. 25, 1912).—Eight to ten partially cured kernels of each of the following varieties were inoculated with macerated pycnidia by a slight abrasion of the surface: Schley, Curtis, Nelson, Teche, Alley, Pabst, and Van Deman. Check kernels of each variety were carried throughout the experiment. Similarly, Teche and Van Deman kernels were inoculated with the two strains of yellow bacteria (strains 100 and 104). After five days the bacterial inoculations had caused a softening of the tissues throughout, but there were no evidences of the kernel spot. The fungous inoculations had in nearly every case taken, and spots typical of the disease both externally and internally had developed, regardless of variety. The checks were sound, except for an occasional contamination with *Botrytis cinerea*, which had caused a general softening of the tissue. Reisolations of the fungus were made from each of the varieties inoculated, and one of these strains was used in the next experiment.

EXPERIMENT NO. 6 (Jan. 6, 1913).—Three Petri dishes of partially cured Van Deman kernels were inoculated upon the slightly abraded surface with macerated pycnidia of the fungus reisolated from artificial inoculation in experiment No. 6. Three dishes of kernels were similarly inoculated with a *Sphaeropsis* obtained from old decaying pecan hulls, while two were held as checks. Observations after five days showed infection with typical symptoms in every case of inoculation with the kernel-spot fungus. The *Sphaeropsis* had caused a general breakdown and softening of the tissues, with slight discoloration, but with no symptoms like the disease in question. The checks all remained sound and free from infection of any kind.

No opportunity for field inoculations has presented itself without the accompanying danger of introducing or spreading the disease, and hence the infection tests have been entirely confined to the laboratory. However, the characters of the disease are so definite and the results of the inoculation work on kernels in the laboratory have been so largely positive that the fungus tested (strain 99 and its reisolation) may now be legitimately regarded as the cause of the kernel-spot. The general disorganization and moist softening of the tissues brought about by the bacteria and by the *Sphaeropsis*, *Botrytis*, and *Penicillium* fungi was entirely different in appearance and result from the disease under investigation. Individual infections of the latter occur within limited and well-defined boundaries and, though giving a pithy consistency to the diseased parts, never cause a moist softening of the injured tissue.

## CULTURAL STUDIES

As grown upon corn-meal agar the optimum temperature for the fungus was found to lie around 20° C. (68° F.). No growth took place below 2° or above 37°. The rate was slow at 4°, but gradually increased up to the optimum, and decreased somewhat more rapidly in rate above that point. At 35° a slight but abnormal growth occurred for a few days, but at the end of the 3-weeks' test, incubation at the optimum temperature failed to show any further signs of life in these cultures.

Upon corn-meal agar the submerged growth varies but little from a sepia brown, while the aerial mycelium shows gradations from that to whitish. Usually a large number of dark-sepia to almost black pycnidia are formed upon this medium. The mycelium is straight and but little branched, with gradations from brown to almost hyaline.

On corn-meal flasks the colonies appear very much as upon the corn-meal agar, though the aerial mycelium is usually much more luxuriant and cottony, becoming, however, somewhat felted with age. Pycnidia are developed in large numbers.

On cooked-potato cylinders the colonies are brown ocher, varying also to a slightly darker shade. The surface is smooth and glistening, becoming somewhat wrinkled with age. No aerial mycelium or pycnidia have been observed on this medium. The cells of the hyphæ differ from those grown upon corn-meal agar in being more nearly isodiametric, with thicker and somewhat bulging walls. The mycelium possesses but few side branches, and the color varies from pale brown to almost hyaline. In cultures several weeks old the whole potato cylinder becomes somewhat softened and turns brown, but no fungous mycelium is found except near the surface. The starchy contents of the potato cells become largely digested, though the walls of the deeper lying cells remain intact except for the breaking down of the middle lamellæ.

Upon synthetic agar the growth is brown ocher to sepia in the older and drier parts. The surface growth often becomes more or less wrinkled and moist-mealy in appearance in older cultures, while a pale brown to whitish aerial mycelium may or may not develop. Microscopically the hyphæ very much resemble those developed upon the potato cylinders, but the thickening and bulging of the walls is often much more apparent. Indeed, the hyphæ frequently break up into their constituent cells, and it is this behavior that gives the moist-mealy appearance to some cultures.

## MORPHOLOGY AND TAXONOMY

The study of this fungus in culture and upon the host has shown it to conform in characters with the genus *Coniothyrium*. However, no member of this genus has been found hitherto reported on the pecan or any nearly related host. It thus becomes necessary to give the fungus a new specific value until cultural and cross-inoculation work can establish

its connection with a previously described *Coniothyrium* occurring upon some widely differing host. An enumeration of the characters thus far observed is given below.

It should be stated that the pycnidia have been observed mostly in culture, their formation on the host having been confined to the extracted kernels in a damp chamber. In the latter case their development has taken place at or near the surface of the kernel and often accompanied by a thin subicle of brown to whitish hyphæ.

***Coniothyrium caryogenum*, n. sp.**

Upon pecan kernels *Coniothyrium caryogenum* causes dark-brown, irregularly roundish surface spots with a hemisphere of pithy tissue beneath, which is surrounded by a brownish layer of host cells.

Mycelium brown, sometimes almost hyaline where not submerged, septate, slightly branched, straight or within the host cells often separating into the constituent hyphal cells which are then more or less swollen and thick walled.

Pycnidia roundish, osteolate, thin walled, dark brown, about 200 to 250  $\mu$  in diameter.

Sporophores short and indistinct. Spores pale brownish, elliptical, 1-celled, 2.5 to 3.6 by 1.8 to 2  $\mu$ .

Habitat.—Kernels of *Carya illinoensis* (Wang.) K. Koch. Type specimens from large seedling tree belonging to Mr. James R. Vann, Thomasville, Ga. Specimens also received from Raleigh, N. C.; Baconton, Ga.; Monticello, Fla.; Minden, La., and other points in the pecan belt, including Texas.

CROWN-GALL

[Caused by *Bacterium tumefaciens* Sm. and Town.]

So far as known, the crown-gall has not hitherto been published as occurring on the pecan from natural infection. However, in the fall of 1909 specimens of young trees affected with both the hard and soft types of galls (Pl. XXXVI) were received from a nursery in Mississippi with the statement that about 0.1 per cent of the stock in the nursery was infected. The disease has also been observed by the writer at one point in northern Florida. But, since these two localities have furnished the only cases reported, it may be considered as of very rare occurrence upon this host.<sup>1</sup>

On the pecan the tumors occur not only at the collar of the tree but several inches higher up on the stem and also on the roots. The greater prevalence of the disease near the surface of the ground is explained by the fact that the parasite first enters the host tissues through wounds. Thus, the process of grafting and the subsequent treatment of the stock readily furnish conditions requisite for infection and further development. The typical appearance of the disease may be inferred from the name; the galls at first consist of a succulent growth of the young host cells thrust out from the cambium layer in the form of a tumor which may attain a considerable size. With age the surface becomes much

<sup>1</sup> The only practical method of control hitherto employed consists in rigid nursery inspection. Obviously, no trees showing the disease should be planted, even though the pecan does not appear to be as seriously affected as many other plants.

roughened and darker in color and the interior tissues are then more or less distorted and hardened. Often the interior assumes a distinctly woody texture, and a roughened bark develops over the surface to form the "hard-gall" type. With the development of roots from the tumor tissue the "hairy-root" type appears, but this form has not been observed on the pecan.

#### EXPERIMENTS WITH THE CROWN-GALL ORGANISM

Soft galls from the Mississippi nursery (December, 1909) were left for five minutes in a solution of corrosive sublimate (1:500) and washed in sterile distilled water. Small pieces of the abnormal tissue were then removed under aseptic conditions from points just under the surface and near the edge of the galls, and beef-agar cultures started by the ordinary poured-plate method. In from three to eight days the circular and somewhat opalescent colonies of the organism appeared, but were much more abundant in cultures started from the extreme base of the young soft galls near the juncture between the diseased and healthy tissues. Transfers were made to beef-agar slant tubes, and with one of the strains thus obtained the following inoculation tests were made.

EXPERIMENT NO. 1 (December, 1910).—Six table beets were inoculated by needle punctures from young beef-agar cultures of the bacteria, while a like number of beets were punctured with sterile needles and held as checks.

After five weeks, examination of the inoculated beets showed the development of typical galls, 3 to 10 mm. in diameter, at most of the needle punctures, while the checks showed no signs of infection.

EXPERIMENT NO. 2 (Jan. 12, 1911).—Four potted pecan seedlings were inoculated by scalpel punctures at the crown from 4-day-old beef-agar cultures, and the soil was replaced around the base of the tree to preserve the moist condition. Four other seedlings were treated in the same manner, except that no bacteria were introduced. The trees were all dormant at this time and remained in this condition until the latter part of March, when, with the exception of one of the inoculated trees which died from other causes, all pushed out their foliage in the normal manner.

Examination in June showed a tumor several millimeters in diameter at the crown of one of the inoculated trees and an apparently incipient infection on a second. All the other trees had completely healed over, so that the location of the punctures could scarcely be made out. On September 12, eight months after inoculation, well-developed galls were found at the crown of two out of the three remaining inoculated trees. The check trees, together with 59 other pecan seedlings in the same greenhouse, showed no indications of the disease.

Since these brief studies with the parasitic organism were carried out merely to indicate the connection of this disease of the pecan with the well-known crown-gall, no further inoculation and cultural tests were made. However, cultures of the bacterium were submitted to Dr. Erwin F. Smith, of the Bureau of Plant Industry, who obtained similar results in inoculation experiments and further verified the identity of the organism with *Bacterium tumefaciens* Sm. and Town., the cause of crown-gall of plants.

## SUMMARY

The nursery-blight is a serious disease of young trees, but is rarely found to be injurious in orchards. Its distribution corresponds closely with that of the host. The casual fungus, *Phyllosticta caryae* Peck, attacks only the leaves of the pecan. Infection first becomes evident through the formation of tiny circular, dark-brown spots, which increase gradually in size and finally become grayish white in the center of the upper surface and usually blackish throughout on the lower. Entire defoliation of young trees sometimes takes place. Spraying with Bordeaux mixture has proved a very effective method of control. Since the disease is primarily a nursery trouble, the question of disease resistance would not be applicable in this connection. All attempts at pure-culture inoculation have been successful. A combination of high humidity and temperature seem best to favor the spread of the disease. The fungous mycelium ramifies through the intercellular spaces above the lower epidermis and throughout the mesophyll tissue. Pycnidia are few on the living leaves, but are produced in abundance on some culture media.

The brown leaf-spot usually causes very little injury, but is widely distributed and occasionally during wet seasons some defoliation may result. The fungus *Cercospora fusca*, emend. sp., causes dark reddish brown spots of uniform color on both leaf surfaces. These are at first somewhat angular in outline as bounded by the veins of the leaf, but may later become roundish and more indefinite in their margins. There appears to be little difference in resistance to this disease among the varieties now commonly planted. The rather limited observations upon the effect of Bordeaux mixture were favorable to the control of the disease. Pure-culture inoculations were highly successful, giving the typical disease symptoms. The temperature relations were very similar to those of the nursery-blight. The mycelium is largely intercellular in its growth, but aggregations of fungous cells break through the upper epidermis to bear the pale tawny conidial clusters, and a creeping surface mycelium sometimes occurs. True spore formation has not taken place in culture.

The pecan anthracnose is well distributed, but hitherto has not usually been very serious at any one point. It has been shown by cultural and cross-inoculation work to be due to *Glomerella cingulata* (Stonem.) S. and v. S., the fungus causing bitter-rot in apples. On the leaves infection causes the formation of irregular reddish to grayish brown blotches varying greatly in size and eventually often covering the whole leaf. On the nuts the blotches are also irregular in outline, but nearly or quite black and often slightly sunken below the surrounding healthy tissue. The production of acervuli and perithecia occurs under suitable conditions of temperature and humidity. The problem of control is largely in the tentative stage, though from the work of Scott and others

on the apple bitter-rot it is thought that Bordeaux mixture will prove effective. Some indications of difference in varietal resistance have been observed. High temperature and humidity furnish the optimum conditions for growth and spread of the disease, as is the case with the bitter-rot of apple.

The kernel-spot is fortunately rare, but on this account the present study has been largely confined to laboratory and greenhouse work. The fungus *Coniothyrium caryogenum*, n. sp., causes the development of dark brown to almost black surface spots upon the kernel. Internally the diseased tissue extends in an approximate hemisphere beneath the dark-colored spot and is pithy in texture and bitter to the taste. Pure-culture inoculations have been largely successful. The optimum temperature for growth was found to be about 70° F. The mycelium enters the cells of the kernel, where it is often more or less swollen and broken up into its constituent cells. Pycnidia have been produced abundantly in culture, but on the host only on the extracted kernels in a damp chamber.

Crown-gall has been found on the pecan in northern Florida and southern Mississippi. It is similar in appearance to the well-known crown-gall of plants and has been shown by pure-culture and inoculation work to be due to the same organism, *Bacterium tumefaciens* Sm. and Town.



## DESCRIPTION OF PLATES

PLATE XXXIII. Fig. 1.—Pecan nuts infected with the anthracnose fungus by spraying with a distilled water suspension of conidia, showing the appearance nine days after inoculation. Natural size. Fig. A.—Four check nuts, two punctured with sterile needle and two unpunctured. Fig. B.—Four nuts inoculated upon the unpunctured surface of the hull. Fig. C.—Four nuts inoculated after puncturing the surface of the hull with a sterile needle.

Fig. 2.—Three of the infected nuts shown in figure 1 after further development of the acervuli.  $\times 1\frac{1}{2}$ .

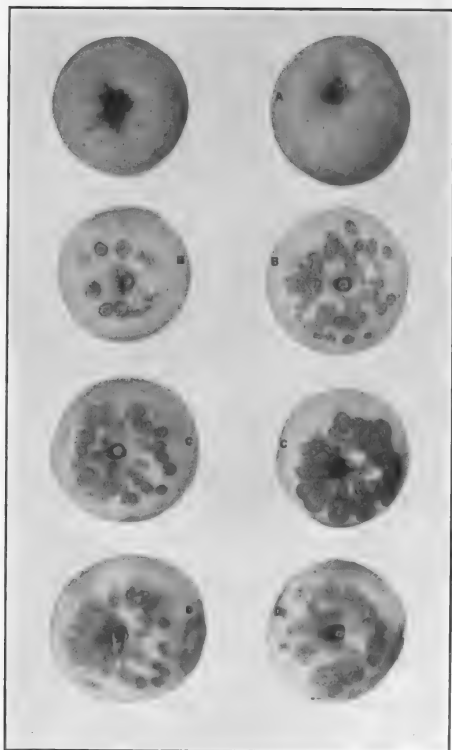
XXXIV. Yellow Newtown apples infected by needle puncture with conidia of the anthracnose fungus from pecan and apple, showing appearance four days after inoculation. One-half natural size. Fig. A.—Check apples punctured by sterile needle. Fig. B.—Apples infected by needle punctures with strain 150 from the apple. Fig. C.—Apples infected with strain 123 from a diseased pecan hull. Fig. D.—Apples infected with strain 125 from a diseased pecan hull.

XXXV. Yellow Newtown apples infected by needle puncture with conidia of the anthracnose fungus from pecan and apple, showing appearance four days after inoculation. Two-thirds natural size. Fig. A.—Check apple punctured by sterile needle. Fig. B.—Apple infected with strain 125 from the pecan nut. Fig. C.—Apple infected with strain 123 from the pecan nut. Fig. D.—Apple infected with strain 150 from the apple. Fig. E.—Apple infected with strain 146 from the pecan leaf. Fig. F.—Apple infected with strain 158, a reisolation of strain 125 after passage through the apple.

XXXVI. Crown-gall (caused by *Bacterium tumefaciens* Sm. and Town.) on pecan nursery trees from southern Mississippi. Natural infection. Two-thirds natural size. Fig. 1.—The soft type of gall. Fig. 2.—The hard type of gall.

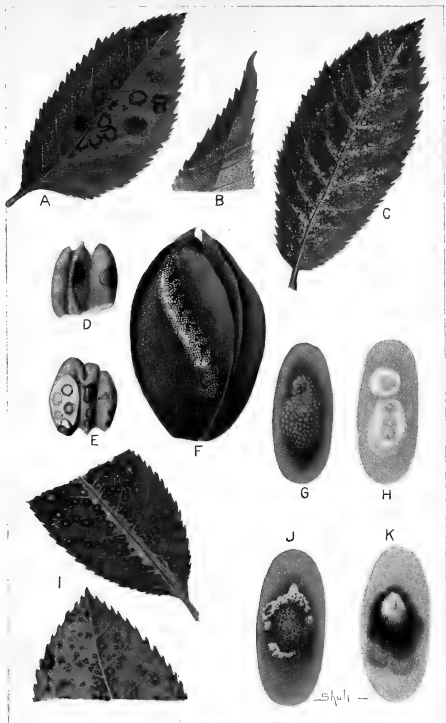
XXXVII (colored). Fig. A.—A pecan leaflet infected with the brown leaf-spot fungus (*Cercospora fusca*, emend. sp.) from pure culture. Fig. B.—A pecan leaflet infected with the anthracnose fungus (*Glomerella cingulata* (Stonem.) S. and v. S.) from pure culture. Fig. C.—View of upper surface of a pecan leaflet recently infected with the nursery-blight fungus (*Phyllosticta caryae* Peck) from pure culture. Fig. D.—A pecan kernel infected with the kernel-spot fungus (*Coniothyrium caryogenum*, n. sp.) from a pure culture, showing the appearance eight days after inoculation. Fig. E.—A pecan kernel with the kernel-spot from natural infection. Fig. F.—A pecan nut infected with the anthracnose fungus from pure culture. Fig. G.—The nursery-blight fungus upon synthetic agar after two weeks. Fig. H.—The nursery-blight fungus on corn-meal agar after two weeks. Fig. I.—Views of the upper and lower surfaces of pecan leaflets, showing an advanced stage of the nursery-blight. Natural infection. Fig. J.—The brown leaf-spot fungus on synthetic agar after four weeks. Fig. K.—The brown leaf-spot fungus on corn-meal agar after four weeks. (All figures are natural size.)











# A TWIG BLIGHT OF *QUERCUS PRINUS* AND RELATED SPECIES

By DELLA E. INGRAM,

*Scientific Assistant, Investigations in Forest Pathology, Bureau of Plant Industry*

## INTRODUCTION

A twig blight of the chestnut oak (*Quercus prinus* L.) was first reported to the Office of Investigations in Forest Pathology on May 31, 1911, by Drs. Metcalf and Spaulding, of that office. Specimens were collected and sent in from York, Pa. Since that time the disease has been reported and diseased specimens have been received from various points throughout Virginia, West Virginia, Maryland, Pennsylvania, New York, and Connecticut. It is not possible at this time to determine definitely the exact range of the blight, as sufficient data have not been obtained. Nothing is known regarding the origin, age, or directions of distribution of the causal fungus, but apparently it will seriously lower the silvicultural status of the chestnut oak.<sup>1</sup>

## EFFECT ON HOST

This blight is primarily a disease of the chestnut oak, but occasionally the American chestnut (*Castanea dentata* (Marsh) Borkh.) and the white oak (*Quercus alba* L.) are attacked. Inoculations in the greenhouse have proved that a number of other species of oak are also susceptible.

Trees of all ages and sizes may be attacked, but usually only the small branches of the larger trees are affected. In some cases where young saplings are attacked the whole tree is killed outright. On the affected twigs the leaves wither suddenly without yellowing, gradually shrivel, and turn a chocolate brown. This browning of the leaves and twigs gives the tree the appearance of the well-known fire-blight of the pear and the apple. (Pl. XXXVIII.) The fungus often stops at the point where the secondary shoots join the main stem, and, as a result, the affected twig may rot at the base and fall off. On the diseased twigs are numbers of small black pycnidia erumpent through the bark. These are sometimes arranged singly and sometimes grouped. Careful sections were made of leaves from diseased twigs brought in from the field, but no mycelium could be found in the tissues. Cultures were also made, but nothing developed. A microscopic examination of a transverse section of the wood reveals the presence of abundant mycelium in the

---

<sup>1</sup> Apparently the only practical method of control for individual trees is cutting back the young twigs several inches below the darkened portion. However, under forest conditions no practicable means of control is known.

tracheary tubes and throughout the cells of the inner and outer bark. A study of the distribution of the mycelium in the twigs of different ages and the relative amount present in the wood and cambium of the diseased twigs was not undertaken.

#### MORPHOLOGY OF FUNGUS

Soon after the leaves wither on the affected twigs, small papillæ begin to form under the bark, which in the course of a few weeks break through

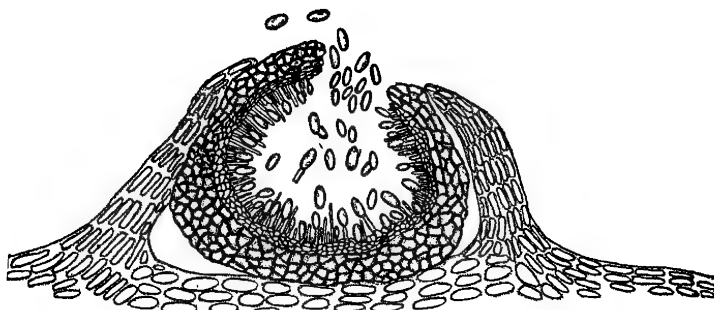


FIG. 1.—*Diplodia longispora*: A section of a pycnidium.

in the form of the small, black pycnidia mentioned above. These are globose to subglobose in shape, very distinctly ostiolate, and dark brown to black in color. In size they vary from  $95$  to  $145\mu$  in diameter. In cross section (fig. 1) the wall of the pycnidium is made up of practically two parts: The outer, dark carbonlike layer and an inner membranous layer of typical fungous cells. These cells have a decidedly purplish tinge, merging into hyaline as the s orogenous layer is reached.

The spores both on the host and in culture are oval or ovoid (fig. 2, A), often tapering somewhat at one end, densely granular, often very thick-walled, averaging about  $29 \times 11\mu$  in size. At first the spores are hyaline and continuous, but after some time (fig. 2, B) they take on a yellowish tinge and finally become dark brown in color and 1-septate. Rarely the septum forms in the hyaline spores before the color begins to change, but this is not usually the case.

The spores are borne singly on rather short, broad conidiophores, interspersed with numerous filiform paraphyses, and are abjoined from the tip at maturity by a constriction near the end of the conidiophore. The conidiophores may become long and filiform in artificial media. The liberation of the spores from the pycnidium is effected in damp weather by means of distinct cirrhi, or threads, forced out through the ostiole of the pycnidium.

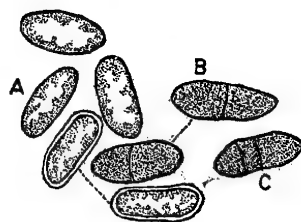


FIG. 2.—*Diplodia longispora*: Stages in development of spore. A, Macrospoma stage; B, Diplodia stage; C, Diplodia spore with two septa.



## INOCULATIONS

Inoculations were carried on in the greenhouse on *Castanea dentata* (Marsh) Borkh. and on a number of related species of oak—*Quercus prinus* L., *Q. minor* (Marsh) Sarg., *Q. gambelii* Nutt., *Q. lobata* Nee., *Q. texana* Buckl., *Q. virginiana* Mill., *Q. alba* L., and *Q. rubra* L.

At the time of the first inoculations small potted trees were used, and these were mostly in their dormant winter condition.

The inoculations were made by sterilizing the bark with a mercuric-chlorid solution, making an incision through the bark with a sterile scalpel, and carefully inserting a portion of the mycelium. The wound was then carefully protected by a small portion of sterile cotton. Check plants were kept of all inoculations made.

The first inoculations were made on chestnut on October 24, 1911, as no chestnut oak was then available. In seven days the inoculated twigs showed a darkened area in both directions from point of infection. After one month the twigs were entirely dead from the point of inoculation outward, and the small papillæ of the fungus were visible just beneath the epidermis. The checks healed normally.

A pure culture of the fungus was obtained from a portion of a diseased twig that was brought into the laboratory. From this culture inoculations were made on November 11, 1911, as follows:

Four inoculations on *Quercus lobata*, two by means of an incision in the bark and two by simply binding on portions of mycelium in agar with sterile cotton; three inoculations on twigs of *Castanea dentata*; and three inoculations on leaves of *Q. prinus*. One leaf of *Q. prinus* was inoculated on the upper surface through the wounded epidermis and one on the lower; on the other, the mycelium was simply spread over the unwounded surface.

An examination after one week showed inoculated twigs of *Quercus lobata* blackened for about half an inch each way from the point of inoculation; the chestnut was slightly darkened. The wounded leaves of *Q. prinus*, both inoculations and checks, were somewhat yellowed, but these subsequently recovered; the unwounded inoculated leaf was normal; and all were uninjured by the fungus. After some weeks these leaves were brought into the laboratory and careful sections made, but no trace of the mycelium could be found in the tissues.

In all, a total of over 50 inoculations were made in the greenhouse to test the susceptibility of different species of oak and to find the time when infection most readily takes place. Of these inoculations 50 per cent were effective. The twigs darkened and the leaves withered, showing the presence of the fungus. In some the infection did not extend more than a few inches from the tip, but in others the whole twig died. In but few cases, however, did the fungus make its way into and up the main body of the plant.

*Quercus gambelii* proved to be the most susceptible when inoculated, and *Q. lobata* the second; *Q. alba* and *Q. rubra* were slower in showing the effects of the fungus; while *Q. virginiana* and *Q. texana* were not affected.

In a number of cases the plant was in a dormant condition when inoculated and seemed not to be affected by the fungus, but at the leafing-out season no leaves were formed from the point of inoculation outward to the end of the branch (Pl. XXXVIII), while the other part of the plant put out leaves and grew in a normal manner. After inoculation the twig darkened slightly, but no further external development took place. No pycnidia were formed as usual, even after the growing season commenced.

The failure of part of the inoculations was probably due to the time of inoculation, as it was found that the twigs are the most susceptible when the new shoots are just coming out. Practically all the inoculations made at this time were effective, but after two weeks from the time of leafing-out the susceptibility lessened greatly, only a small percentage made from that time on having any effect.

In some cases after the dying of the tip the branch put out new shoots below and apparently overcame the injurious effect of the fungus. Inoculations from cultures of the mature stage developed somewhat slower than those from the *Macrophoma* stage.

The inoculations of *Quercus prinus* in the field were more conclusive. Fifty inoculations were made on May 8, 1912, and 28 of these were effective. Twenty-six were made in the usual manner by a slight incision in the bark and the inserting of a portion of the mycelium into the wound. Fifteen were made by inoculating with spores. Of the latter, 10 were made by placing the spores in the incision and 5 by puncturing the bark with a needle and spraying the injured part with spores suspended in corn-meal infusion. Four inoculations were made by binding the mycelium on the surface of the uninjured twigs. Five leaves were pricked slightly with a needle and sprayed with the spores—one on both upper and lower surface, two on the upper surface only, and three on the lower only. Checks of both leaves and twigs were treated in the same manner. The leaves all healed normally and were not affected by the fungus. Three of the twigs that were sprayed with spores withered and died, while the two others healed normally. Four of the twigs inoculated with spores by a slit in the bark withered from the point of infection out to the tip; the others were uninjured by the fungus and put out new leaves and shoots. Of the 26 twigs inoculated with mycelium on wounds, 21 showed the effects of the fungus, most of them dying completely from point of inoculation outward; those unwounded showed no effects whatever but grew in a normal manner. The inoculations were made partly on small saplings and partly on the small branches of larger trees. The largest sapling which died com-

pletely was about 8 feet high and the main trunk about  $1\frac{1}{2}$  inches in diameter. After two weeks the ends of the twigs withered and the leaves dried up. The twigs showed the darkening of the cambium for a distance of 6 inches from the tip. Sections across the twig also showed pustules of the fungus just beneath the bark. After three months the pycnidia had broken completely through the bark, spores of both types being present in the pycnidium. On June 1, 1912, 20 other inoculations were made in the field by the wounding of the bark and inserting a portion of mycelium. Checks were treated in like manner. Of these only 7 were effective, as the twigs were by that time older and possibly more resistant. In no case were there any large limbs killed, only the small branches and tips.

#### CULTURE WORK

The fungus grows well in culture, but does not fruit readily, and then only on solid media. Fresh twigs of *Quercus alba* and *Q. prinus* were brought in from the field and sterilized by wiping with mercuric-chlorid solution and rinsing with distilled water. The bark was then pricked in several places, and portions of agar containing mycelium were spread over these portions. These were then put in test tubes with sufficient moisture. In one week discolored areas appeared on the twigs, and in three weeks the small black pustules of the fungus appeared. On examination these proved to be the *Macrophoma* stage.

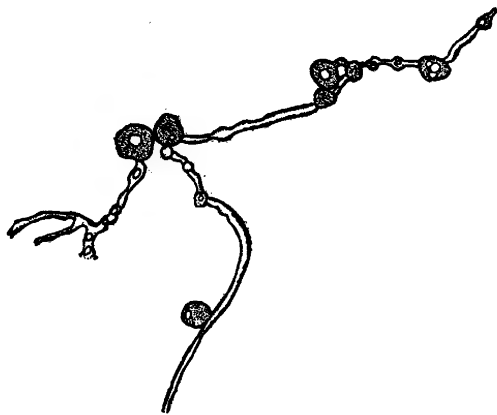


FIG. 3.—*Diplodia longispora*: Sclerotial bodies formed in artificial media.

Twigs of the same species were also used, sterilizing them by the use of the autoclave. The growth on these was almost entirely superficial, the mycelium completely covering the twigs in a grayish green, felty mass. Occasional humps or tufts of mycelium were present in which a few pycnidia containing spores of the *Macrophoma* type were found. After six months no further development had taken place. As a medium the autoclaved twigs proved to be much inferior to the unheated twigs.

Of the agars corn meal and prune gave the best vegetative growth and were used to the exclusion of others in securing pure cultures and in germination studies. Portions of the mycelium were transferred to corn-meal flasks or other solid media to secure the formation of pycnidia.

A number of different kinds of media were used: Potato, prune, beef, and corn-meal agars, - 15; potato and beef agars, + 15; corn-meal and

prune agars, +II, Fuller's scale; Raulin's fluid, malt, and string-bean agars; and cylinders of Irish potato, sweet potato, parsnip, and carrot, banana, orange, prune, and apple.

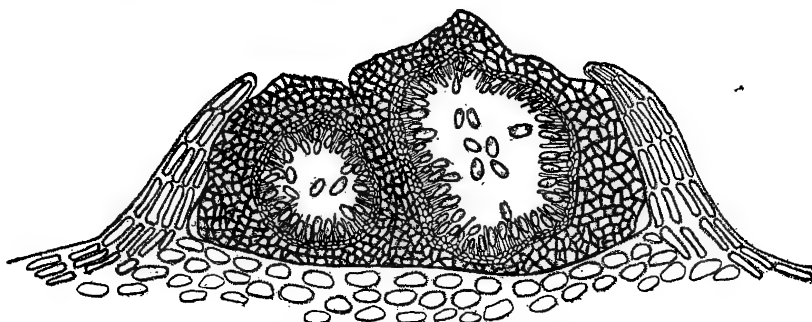


FIG. 4.—*Diplodia longispora*: A section showing grouping of pycnidia.

The Irish potato and the sweet potato gave the best results for the vegetables. The fruits gave an abundance of mycelial growth, but few

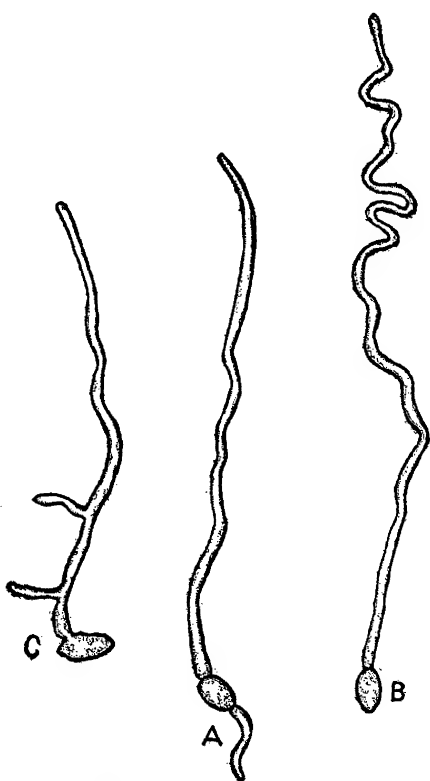


FIG. 5.—*Diplodia longispora*: Types of germination. A, B, Germ tubes from end of spore; C, germ tube from side of spore.

pycnidia. In several media, especially apple, peculiar sclerotial bodies (fig. 3) were formed in abundance. An extremely acid or extremely alkaline medium was not as satisfactory as a nearly neutral one, and starchy media in general gave the best results. On all artificial media which produced pycnidia, a dense stroma was produced and the spores were borne in locules in the stroma. This is not the case on the host, where, while the pycnidia are usually grouped (fig. 4), a typical stroma is never present. On all media the colonies are at first hyaline, later becoming grayish green, and finally almost black.

#### GERMINATION STUDIES

The spores germinate readily in distilled water, corn-meal infusion, Raulin's fluid, and corn-meal, prune, or potato agar. If a diseased twig is placed in a damp chamber many spores will germinate inside the pycnidium. When placed in a liquid medium without being subjected previously to a moist atmosphere, the time varies from three to six hours.

Usually the germ tubes are sent out from the long axis of the spores (fig. 5, *A* and *B*) and occasionally from the sides (fig. 5, *C*). As many as six tubes have been observed from a single spore.

At first the tubes are nonseptate, but the cross walls gradually begin to appear in from two to five days from time of germination. The hyphæ show a marked tendency to coalesce (fig. 6), and

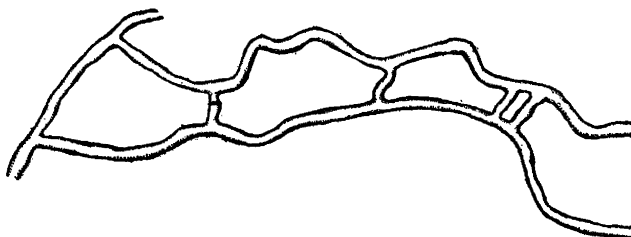


FIG. 6.—*Diplodia longispora*: A portion of mycelium showing the coalescing of the hyphæ.

often unite to form meshes. Soon after the formation of septæ the mycelium begins to darken, taking on a grayish green hue. The hyphæ become constricted, and peculiar chlamydosporelike bodies are formed



FIG. 7.—*Diplodia longispora*: A portion of mycelium with chlamydosporelike bodies.

(fig. 7) intercalary in the hyphæ. When a number of spores are sown at one time, some of them undergo a further development, instead of germinating as

above described. The spore turns a dark olive brown in color, and a central, transverse septum is formed. Occasionally two septæ are present (fig. 2, *C*), but this is not typical.

#### DETERMINATION OF THE FUNGUS

In order to determine definitely whether the *Macrophoma* and *Diplodia* types of spores were really stages in the life history of the same fungus, a number of single spores of each were planted in agar plates, and carefully marked colonies of each from single spores were then transferred to corn-meal flasks. Each first produced the *Macrophoma* stage and later the *Diplodia* stage. Numbers of diseased twigs were brought in from the field and carefully examined the following winter after being attacked, in the hope of finding a perfect stage, but without success. According to Saccardo, this fungus should be called a *Botryodiplodia*, as the pycnidia are usually grouped. However, since the characters which separate it from the genus *Diplodia* may be produced artificially on culture media and vary with the amount of moisture present, it seems advisable to place it in the latter genus.

A number of species of *Diplodia* have been described on *Quercus*, mostly from European countries. All of them are described either from the immature stage, or insufficient morphological characters are given for a positive identification, the spore measurements in several being absent. Only one species has been found described from America—*Diplodia longispora* C. and Ell. on *Quercus coccinea* from New Jersey. It is the

only species which is described with mature spores and in which the spore measurements are given. The morphological characters given agree very well, but, according to the measurements given, the spores are uniformly longer and narrower, being 30 to 35 by  $7\mu$  in comparison with 23 to 32 by 8 to  $12\mu$  of the species under discussion.

However, since there is much variation in this genus and since the perfect form of this fungus may eventually be found, the species herein described is referred to *Diplodia longispora* C. and Ell. While, as mentioned above, the spore measurements do not exactly agree, the variation being considered by some sufficient to warrant a new species, it was not thought desirable to add another species to the already cumbersome and much confused nomenclature of this genus. None of the species described are recorded as causing any disease of the host.

#### SUMMARY

A fungus which is referred to *Diplodia longispora* C. and Ell. is the cause of a destructive twig disease of *Quercus prinus*, also of several other species of *Quercus* and of *Castanea dentata*.

Large trees are not killed outright, but they may eventually die as a result of the weakened condition caused by losing the young branches, and particularly the cumulative effect of the attacks of several years. Saplings are often killed outright.

Infection takes place through wounds in the bark and will not take place through an unbroken surface. The fungus does not extend into the leaves, as no mycelium is present in the leaf tissues.

#### DESCRIPTION OF PLATE

PLATE XXXVIII. An oak (*Quercus gambelii*) inoculated with *Diplodia longispora* at X when dormant. No leaves developed above the point of inoculation.



## NEW POTATO WEEVILS FROM ANDEAN SOUTH AMERICA

By W. DWIGHT PIERCE,

*Agent and Expert, Investigations of Insects Affecting Southern Field Crops, Bureau of Entomology*

During the year 1913 a number of shipments of South American potatoes for experimental propagation by the Department of Agriculture have been intercepted by Messrs. E. R. Sasscer and H. L. Sanford, inspectors of the Federal Horticultural Board, because of more or less serious infestations by weevils. In most of the shipments the weevils were alive. Those received early in the summer were partly immature, while in later shipments they were all mature. When the material was shipped it was supposedly free of insect pests, and in fact it is quite possible to find a potato apparently whole which contains a weevil within. Mr. C. H. T. Townsend, the Entomologist of Peru, writes that the work of the weevils is often undetected until the potatoes are cooked and served on the table. It can therefore be seen how readily a shipment of South American potatoes received for planting purposes might be passed by quarantine officers and perhaps be the source of a very dangerous pest to the American potato industry.

As a result of the finding of weevils in many shipments of potatoes, the Federal Horticultural Board has taken action excluding South American potatoes from the United States. This article has therefore been prepared with the view of assisting the inspectors in their work and also to place on record descriptions of the weevils in question.

The three species of weevils so far found are very different in appearance and can be readily identified from the illustrations published herewith.

A notice of the finding of a species of weevil known as *Rhigopsidius tucumanus* Heller in potatoes shipped by Mr. W. F. Wight from points in Peru, Bolivia, and Chile has been published.<sup>1</sup> Since the publication of this note two other species, each representing a new genus and a new species, have been discovered.

The second species found in shipments of potatoes from Peru was obtained alive on July 9, 1913, by Mr. Sasscer in a potato sent by Mr. Wight from the mountain districts of Peru. The adult weevil was found just under the skin of the potato in a small cell which had evidently served as a feeding cell for the larva. From the material received it is judged that the larva does not bore extensively in the potato.

<sup>1</sup> Sasscer, E. R., and Pierce, W. Dwight. Preliminary report of the finding of a new weevil enemy of the potato tuber. *Proc. Ent. Soc. Wash.*, v. 15, no. 3, p. 143-144, pl. 4-5, Oct. 2, 1913.



This weevil (Pl. XLI, figs. 1 and 2; and text figs. 1 and 2) forms the type of a new genus in the family Brachyrhinidæ, subfamily Entiminae, tribe Ophryastini, to which our North American genera Ophryastes, Eupagoderes, Amydrogmus, and Tosastes belong. In Lacordaire's group "Leptopsides vrais" it is to be placed near Bastactes and Catasarcus, from both of which it differs by many characters. The descriptions which follow will serve to identify it.

**PREMNOTYPES**, new genus.

Name derived from *πρέμων* (root) and *τρύπω* (to bore), meaning a root borer. Type of genus.—*P. solani*, n. sp.

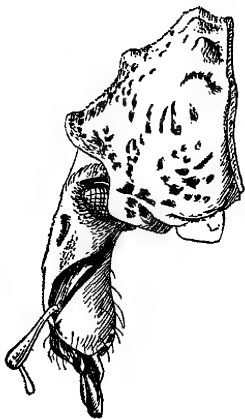


FIG. 1.—*Premnotypes solani* Pierce: Lateral view of prothorax and beak.

Upper surfaces roughly sculptured throughout and closely squamose. Beak longer than head, enlarged at alæ, more or less distinctly depressed on the median line and at the sides; scrobes broadened behind and then flexed downward far from eyes; mandibles beneath not acutely toothed. Eyes vertical, elongate oval, pointed beneath. Antennæ with scape clavate, not greatly overlapping the anterior edge of the eyes; funicle 7-jointed, with first two joints elongate, the others shorter but not transverse; club elongate oval. Prothorax very tuberculate above and at sides; anterior lobes without vibrissæ, almost completely covering the eyes; base truncate, apex convex. Elytra with humeri rounded; striation irregular, with alternate intervals multituberculate. Body wingless. Thorax beneath with all parts short; mesothoracic side pieces unequal; metepimera broad. Intercoxal process broad; first two abdominal segments occupying over half the abdomen; first suture arcuate; second segment at least as long as the two following; fifth segment as long as the two preceding. Femora and tibiæ stout; tibiæ mucronate; tarsi with third joint bilobed and a little wider than the preceding joints, pubescent beneath; claws simple. The posterior tibiæ have the point of attachment of the tarsi terminal and close to the mucro. The apical surface is divided by a ridge into two unequal disks, the inner being the larger. The ridge passes just outside of the corbel.

***Premnotypes solani*, n. sp.**

Length, 7 mm.; breadth, 3.75 mm. Color brown, with bronzy scales.

Beak longer than head and narrower than eyes, being narrowest at about the middle, where the flare of the scrobes begins to widen it. Alæ strongly flared, making apical portion of scrobes open above. Head with small tubercles above the eyes. Median line sharply defined, deepened at frontal fovea, then bifurcate to form a median ridge. The fine median line begins again on this ridge and extends to the apex.

Beginning even with the front edges of the eyes the lateral impressions extend half the length of the beak. Apex of beak shining black, raised in an arcuate band, which causes the shining semielliptical nasal plate to stand obliquely. Mandibles shining black, with at least two inner teeth and with a long, shining, acute, deciduous piece with sharp inner edges. The right-hand deciduous piece has a tiny tooth on the inner edge before the middle. Antennal scrobes strongly flexed downward; scape clavate; funicle with all joints longer than wide, gradually decreasing in size toward

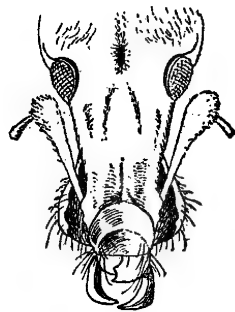


FIG. 2.—*Premnotypes solani* Pierce: Frontal view of beak.

apex; club elongate, with the first two joints occupying over half the bulk. Head, beak, and scape densely clad with fine, silky, bronzed scales; funicle sparsely pubescent; club minutely pubescent.

Prothorax basally truncate, apically sinuate, strongly lobed over eyes, lobes without vibrissæ; coarsely punctured, finely squamose with yellowish to golden metallic scales; median line punctate, strongly impressed; surface with six basal, two discal, and four apical tubercles; widest behind middle at points of lateral basal tubercles.

Elytra at base no wider than thorax; humeri rounded; sides rounded, rough, wider than prothorax. Scutellum minute, triangular, depressed. Surface densely minutely scaly; striæ irregular, with small definite punctures; entire surface rough, but the third, fifth, and seventh intervals especially are raised by a series of small tubercles, which give the striæ a wavy direction.

Prosternum strongly arcuately emarginate, not more than one-half as long as pronotum. Anterior coxæ contiguous. Mesosternum taken up almost entirely by the coxæ, which are narrowly separated; side pieces unequal. Metasternum also short. Undersides and legs densely squamose.

Type.—Cat. No. 16689, U. S. National Museum.

The third species also belongs to a new genus quite closely related to *Premnotypes* and belonging in the same tribe. Several specimens in a more or less perfect condition were found by Mr. Sanford in cells in potatoes received October 9, 1913, from Cuzco, Peru. This species breeds in a manner closely resembling that of the *Premnotypes solani*.

This species (Pl. XLI, fig. 3; text fig. 3) may be identified from the following technical descriptions.

#### **TRYPOPREMNON**, new genus.

Name derived from *τρῶναι* (to bore) and *πρέμων* (root), signifying a root-borer. The name is simply "*Premnotypes*" reversed, because the two genera belong side by side. *Type of genus*.—*T. latithorax*, new species.

Upper surfaces roughly sculptured throughout and closely squamose. Beak longer than head, enlarged at alæ, not impressed on median line except at frontal fovea and near apex; scrobes broadened behind and abruptly truncate; mandibles beneath sharply toothed. Eyes vertical, elongate oval, pointed beneath. Antennæ with scape clavate, not greatly overlapping the anterior edge of the eyes; funicle seven-jointed, joints 1 and 2 elongate, the others progressively shorter and the last three transverse, moniliform; club elongate oval. Prothorax very roughly molded; median line deeply impressed; anterior lobes without vibrissæ, almost completely covering the eyes; base truncate; apex sinuate. Elytra with humeri rounded; striation irregular, with alternate intervals rough and raised. Body wingless. Thorax beneath with all parts short; mesothoracic side pieces unequal; metepimera elongate, moderately broad. Intercoxal process broad; first two abdominal segments occupying over half the abdomen; the first suture arcuate; the second segment as long as the two following; fifth segment as long as the second. Femora and tibiæ stout; tibiæ mucronate; tarsi pubescent beneath, with third joint strongly bilobed, the lobes much wider than the preceding joints; claws simple. The posterior tibiæ have the point of attachment of the tarsi terminal and close to the mucro. The apical surface is divided by a ridge into two almost equal slanting disks, like a roof. The ridge runs directly to the middle of the corbel.



FIG. 3.—*Trypopremnon latithorax* Pierce: Lateral view of thorax and beak.

***Tryporemnon latithorax*, n. sp.**

Length, 6 mm.; greatest breadth, 2.75 mm. Beak longer than head and narrower than eyes except at alæ; the dorsal squamose portion being gradually narrowed from the eyes to the apex. Alæ strongly flared, making the apical portion of the scrobes open above. Head very slightly tumid above the eyes. Median line distinct only to the frontal fovea, which is deeply depressed and very faintly indicated beyond this point. The lateral depressions on the beak are quite faint. Apex of beak shining reddish, with the nasal plate polished, ogival, and raised at apex. Mandibles shining, reddish; deciduous piece long, shining, acute, arcuate, with sharp edges and with a strong, acute, erect ventral tooth. Antennal scrobes strongly flexed downward, very much broadened and evanescent behind; scape clavate; funicle with first two joints elongate, the others progressively shorter and the last three transverse, moniliform; club elongate oval. Head, beak, and scape densely clad with fine, silky, bronzed scales; funicle sparsely pubescent; club minutely pubescent.

Prothorax basally truncate, apically sinuate, with very strong supraocular lobes, which are without vibrissæ; coarsely irregularly punctured, finely squamose with golden metallic scales; median line strongly impressed; surface very uneven with two basal and two discal elevations and with the sides very irregular, sinuate or bitumid; widest at posterior lateral tumidities.

Elytra at base narrower than thorax; humeri rounded; sides feebly convex. Scutellum triangular. Surface densely, minutely scaly; striæ irregular, with strong punctures, entire surface rough, but the third, fifth, and seventh intervals especially are raised by a series of tubercles, which give the striæ a wavy direction.

Prosternum strongly arcuately emarginate, hardly half as long as the pronotum. Anterior coxæ contiguous. Mesosternum taken up almost entirely by the coxæ, which are narrowly separated; side pieces unequal. Metasternum also short. Undersides and legs densely squamose.

Type.—Cat. No. 16690, U. S. National Museum.

Differs from *Premnotrypes solani* in the sculpturing of the beak, the shape of the scrobes and mandibles, and of the nasal plate, the absence of distinct tubercles on the head, the shape and sculpture of the prothorax, and the elytral striation. The third tarsal lobes are also much more distinct.

The weevil *Rhigopsidius tucumanus* Heller (Pl. XL) is, according to present information, more widely distributed than either of the other species. It was originally described by Heller<sup>1</sup> from Tucuman, Argentina, and was recorded in the note by Sassocer and Pierce,<sup>2</sup> quoted above,

<sup>1</sup> Heller, K. M. Neue Rüsselkäfer aus Central- und Südamerika. Ent. Ztg. Stettin, 1906. Bd. 67 (Heft 1), p. 7-9, pl. 1., figs. 3, 3a, and 3b.

<sup>2</sup> This weevil (Pl. XL) belongs to the family Psaliduridæ, subfamily Rhytirhininæ, tribe Rhytirhinini. The nearest North American insects are the species of the genus *Thecesternus* in the tribe Thecesternini of the same subfamily.

The following description, taken from Sassocer and Pierce (op. cit.), will identify this species.

Length, 9 mm., yellowish or purplish brown, with thickly matted vestiture of a cinereous shade mottled with black dots. Head concealed from above by prothorax and eyes, almost covered by the lateral prothoracic lobes. Beak moderately short, usually reposing in a deep pocket of the prothorax, which is posteriorly limited by the anterior coxæ. Beak medianly and laterally carinate to a cross carina between the bases of the antennal scapes. Scrobes deep and narrow from apex near tip of beak almost to eyes, then sharply deflected and broader in front of eyes. Scape stout, clavate. Funicle 7-jointed, the last joint apparently a part of the club. Club 4-jointed. Head at base sinuately impressed, with swellings above the eyes. Prothorax very irregularly sculptured but with a deep median furrow widened angularly at middle and also behind. Strial punctation deep but irregular. Intervals tumid behind. Legs stout. Tarsi with third joint not widely bilobed; tarsal claws simple. First and second abdominal segments long; third and fourth shorter than fifth.

in shipments received May 24, 1913, from Mr. Wight, who collected the material at Cuzco, Temuco, and Arequipa, Peru; Oruro, Bolivia, and Ancud or San Carlos and Castro Islands, Chile. In many instances the injury occasioned by these weevils was quite noticeable. A few of the tubers which superficially appeared to be sound were found, on being opened, to be infested with one and sometimes two larvæ or adults. Mr. Sasscer succeeded in keeping two adults alive from May 24 to September 6, during which period they fed but little and then only on foliage of potato. The injury of this species consists of tunnels throughout the potato, as shown in Plate XXXIX, and the work of the two other weevils is very similar.

#### DESCRIPTION OF PLATES

PLATE XXXIX. Injury caused by potato weevils. Fig. 1.—A section of a potato from Peru, showing the larva of *Rhigopsidius tucumanus* in its burrow.

Fig. 2.—A section of a potato, showing the burrowings of *Rhigopsidius tucumanus*. The work of the two other weevils is somewhat similar.

XL. *Rhigopsidius tucumanus* Heller. Fig. 1.—Dorsal view.

Fig. 2.—Ventral view. Both views are much enlarged; natural size, 9 mm.

XLI. Figs. 1 and 2.—*Premnotrypes solani* Pierce (much enlarged; natural size, 7 mm.).

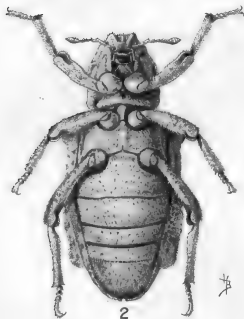
Fig. 1.—Dorsal view. In this drawing the beak, scape, and tibiae are foreshortened, which gives an idea of even greater differences from the succeeding species than really exist.

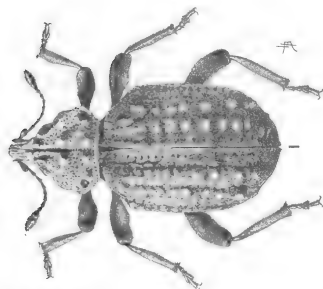
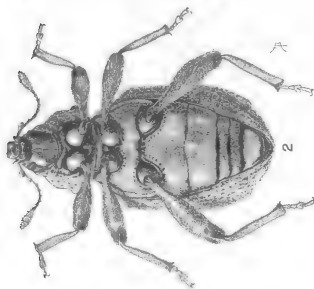
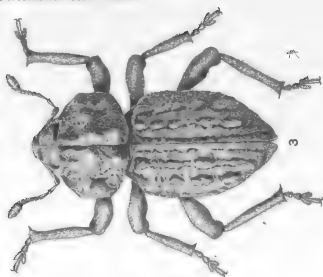
Fig. 2.—Ventral view.

Fig. 3.—*Trypopermnon latithorax* Pierce (much enlarged; natural size, 6 mm.). Dorsal view. In this drawing the scape and the tibiae are not foreshortened as much as in the other species. The different attitude of the beak gives a sense of greater divergence than occurs, as can be seen from the side view of the head and prothorax (see text figs. 1 and 3). The ventral view resembles very closely that of *Premnotrypes solani*.

The drawings which accompany this article were made by Mr. Harry B. Bradford,









# AN UNDESCRIBED SPECIES OF GYMNOSPORANGIUM FROM JAPAN

By W. H. LONG,

*Forest Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry*

## INTRODUCTION

In the Annual Report of the Connecticut Agricultural Experiment Station for 1912 (pt. 5, p. 350), Dr. Clinton reports the introduction of *Gymnosporangium japonicum* Syd. on *Juniperus chinensis*. The rust was found on both stems and leaves of a form known as *J. compacta*, while on a seedling of *J. chinensis* called *J. virginalis* the rust occurred only on the leaves. The plants showing rust only on the leaves were planted in an isolated place. The following spring they were found to be free from rust.

Through the kindness of Dr. Perley Spaulding the writer was able to examine some of the infected material from Dr. Clinton's herbarium containing both types of the rust. The rust on the woody stems seems to be *Gymnosporangium japonicum* Syd., but that on the leaves or young twigs differs in most of its microscopic and macroscopic characters from *G. japonicum*. According to the report, the rust on the leaves or young twigs is apparently an annual, while the other, *G. japonicum*, is a perennial; one is found on the leaves and green twigs, the other on the woody stems; one causes no deformation of the host, the other produces fusiform enlargements 4 cm. in length or longer. The microscopic characters of the two differ as widely as the gross characters mentioned above.

The writer has found in most species of *Gymnosporangium* three types of teliospores in the same sorus. One type has very thick colored walls; one, moderately thick colored walls; and the third, thin and colorless walls. These three types usually differ from each other also in shape and size of the spore as a whole or in the individual cells of each spore. Constant specific characters may occur in one type, often in the thin colorless-walled spores, while they are absent in the other two types or are not so pronounced. For this reason the characters of at least the two extreme types of spores should be given for each species under discussion. In the following descriptions the two extreme types are fully described for two of the species and the three types for the third one. As a matter of convenience in comparing the three species brief descriptions of *G. japonicum* and *G. haraeum* are also given.

## DESCRIPTION OF SPECIES OF GYMNOSPORANGIUM

**Gymnosporangium chinensis**, n. sp.

Æcia unknown.

Telia epiphyllous or caulicolous, appearing on the very small green twigs between the leaves, not causing a fasciation of the young shoots; scattered; usually hemispheric; about 1 mm. in diameter; hazel in color when desiccated.

Teliospores 2-celled; spores with colored walls, oval to broadly ellipsoid, 19 to 22 by 35 to 40  $\mu$  (average for 10 spores, 21 by 36.7  $\mu$ ), slightly but plainly constricted at septum. The two cells are usually subequal; spores rounded at both ends, walls thin, about 1 to 1.5  $\mu$ , pedicel cylindric; pores, one to two in each cell near septum, or rarely only one in upper cell and apical.

Spores with thin colorless walls, ellipsoid, 17 to 19 by 47 to 52  $\mu$  (average for 10 spores, 18 by 49  $\mu$ ), plainly constricted at septum. The two cells are unequal, the lower being from 3 to 7  $\mu$  longer than the corresponding upper cell; apical cell rounded or only slightly narrowed toward apex, lower narrowed toward base; wall thin, colorless, about 1  $\mu$  thick; pores, one to two in each cell near septum, or usually only one in upper cell and apical.

Host plant.—On *Juniperus chinensis* in the Elm City Nursery, Westville, Conn., March 28, 1911, on stock just imported from Japan. In same packet with *Gymnosporangium japonicum* on the same host. From the herbarium of Dr. G. P. Clinton.

**Gymnosporangium japonicum** Syd.

Telia caulicolous on fusiform enlargements, 4 cm. or more long, of the woody stems, irregular tongue or wedge shaped, about 3 mm. or more long, often in rows.

Teliospores 2-celled, occasionally 3-celled; spores with thick colored walls, ellipsoid, 22 to 24 by 48 to 63  $\mu$  (average size for 10 spores 22 by 54.7  $\mu$ ), cells subequal or lower longer and more narrowed at base, not constricted at septum, narrowed at both ends; walls 1.5 to 2  $\mu$  thick, pores near septum, two in each cell.

Spores with thin colorless walls, elliptic fusiform to linear oblong, 16 to 19 by 57 to 70  $\mu$  (average for 10 spores 16.8 by 65  $\mu$ ), walls 1  $\mu$  thick, not constricted at septum; pores, two in each cell near septum.

Host plant.—On *Juniperus chinensis* from Japan.

**Gymnosporangium harae** Syd.

(Sydow, H., and Sydow, P. *Novae fungorum species*—VIII. *Ann. Mycol.*, v. 10, no. 4, p. 405, 1912.)

Telia epiphyllous or caulicolous on the very small green twigs, not causing a fasciation of the young shoots; scattered; hemispheric to short conic; one-half to 1 mm. in size.

Teliospores 2-celled; spores with very thick colored walls, ellipsoid, 25 to 28 by 35 to 44  $\mu$  (average size for 10 spores 25.7 by 39  $\mu$ ), not or but very slightly constricted at the septum; spores rounded or somewhat narrowed at both ends; the two cells subequal or the lower often larger and more narrowed toward the base than the upper one; walls very thick, 3 to 4  $\mu$ ; pores, two in each cell near septum; pedicel cylindrical.

Spores with walls moderately thick and colored, elliptic oblong, 22 to 26 by 48 to 57  $\mu$  (average for 10 spores 23.6 by 52  $\mu$ ), not or but slightly constricted at septum; spores usually much narrowed at both ends; upper cell often with a mammillate apex; lower cell often longer than upper; walls 2.5 to 3  $\mu$  thick; pores, two in each cell near septum.

Spores with walls thin and colorless, oblong to oblong fusiform, 16 to 19 by 48 to 57  $\mu$  (average size for 10 spores 17 by 51  $\mu$ ), rarely constricted at septum; cells subequal, rounded or narrowed at both ends; pores, two in each cell near septum; walls about 1  $\mu$  thick.

Host plant.—On *Juniperus chinensis* from Japan.<sup>1</sup>

<sup>1</sup> This description was made from a portion of the type material which Dr. Sydow kindly sent to the writer.

The three types of spores are described in full, and their diagnostic character can readily be seen when *Gymnosporangium haraeum* is compared with the other two species given in this paper. In *Gymnosporangium japonicum* and *G. chinensis* the spores with thick and moderately thick colored walls for each species are so similar that the two kinds are described as one; therefore, only two types of spores, thick and thin walled, are described for each of these two species. *G. chinensis* and *G. haraeum* are so closely related that the writer would not publish the former as a new species until he had examined the type material of the latter. After a careful examination, however, the conclusion was reached that the two were distinct, as they differ in certain fundamental microscopic characters. These differences are shown in the description given of each species. The most marked difference between these two species is the position of the germ pores in the colorless thin-walled teliospores. In *G. chinensis* they are plainly apical in the upper cell, while in *G. haraeum* they are just as certainly situated only at the septum in both cells.

According to Dr. Clinton, the telia of *Gymnosporangium chinensis* occur on the leaves, but in the very meager herbarium material examined by the writer they arose between the leaves rather than on them. The telia are therefore stated in the above description to be either caulicolous or epiphyllous.

The three types of spores mentioned in the above descriptions are usually more evident in herbarium material than in fresh, as the obstructing colored contents of the spores fade in drying, thus permitting a clearer view of the spore walls.

The value of taking into consideration at least two types of spores, the thick and thin walled ones, is very evident when the corresponding kinds for each species are compared. For instance, the oval thick-walled spores of *Gymnosporangium chinensis*, with equal cells rounded at both ends, are in marked contrast to the ellipsoid, thick-walled spores of *G. japonicum*, with unequal cells sharply contracted at both ends; while the long, narrow, linear-oblong, thin-walled spores, with equal cells of *G. japonicum*, are very different from the shorter thin-walled spores, with unequal cells of *G. chinensis*. Again, many of the thick-walled spores of *G. japonicum* are so sharply attenuated at both ends that they become trapezoid in shape, while the apical cells often have a distinctly mammillated apex. Neither of these characters is present in the thick-walled spores of *G. chinensis*.

Through the kindness of Dr. Shirai the writer has been able to examine some of the material of *Gymnosporangium japonicum* collected in 1900. It was probably a part of the material used by him in his inoculation experiments with this species.<sup>1</sup> The specimens sent consist of two

<sup>1</sup> Shirai, M. Über den genetischen Zusammenhang zwischen *Rostelia koreaensis* P. Henn. und *Gymnosporangium japonicum* Sydow. Ztschr. Pflanzenkrankh., Bd. 10, Heft 1, p. 1-5, pl. 1-2, 1900.

infected branches. One lesion is on a woody stem 6 mm. in diameter; the other is on a much younger branch 1.5 mm. in diameter. No telia were found on the leaves or very young twigs. The telia and teliospores were similar to those found on the woody stems of the imported *Juniperus chinensis* from Connecticut, but had nothing in common with the telia of *G. chinensis*, which were found on the very young twigs and leaves of this imported juniper.

---

ADDITIONAL COPIES of this publication  
may be procured from the SUPERINTEND-  
ENT OF DOCUMENTS, Government Printing  
Office, Washington, D. C., at 25 cents per copy  
Subscription, per year, 12 numbers - - \$2.50

---



# JOURNAL OF AGRICULTURAL RESEARCH

## DEPARTMENT OF AGRICULTURE

VOL. I

WASHINGTON, D. C., FEBRUARY 16, 1914

NO. 5

### THE PRESENCE OF SOME BENZENE DERIVATIVES IN SOILS

By EDMUND C. SHOREY,

*Scientist in Soil-Fertility Investigations, Bureau of Soils*

#### INTRODUCTION

The isolation of organic compounds from soils may have an interest other than that of the purely scientific nature attached to any increase in our knowledge of the composition of soils. This is true, not only when the compounds are known to be readily reactive with other compounds or to have an effect on the microflora of the soil or the growth of higher plants, but also when their constitution indicates that they may have such an effect. Recently three organic compounds have been isolated from soils that seem to be of this nature.

These compounds, rather closely related in constitution, are benzoic acid, metaoxytoluic acid, and vanillin. They were obtained from samples of sandy soil from Florida at present devoted to orange culture. These soils are composed of quartz sand ranging in color from light gray to brown and contain very little organic matter. For the most part this organic matter is deposited in a thin layer on the grains of sand, so that when the soils are treated with dilute alkali and the film of organic material is thereby dissolved or loosened pure white quartz sand remains. The samples, about 90 kilograms in each case, were from eight locations, the top soil and subsoil being represented by separate samples.

#### BENZOIC ACID

Benzoic acid was obtained from but one of these samples—a subsoil. There was no indication of its presence in the corresponding surface soil, and, although indications were obtained of its presence in other subsoils of this series, it could not be isolated in a pure form in sufficient quantity for identification.

The method by which benzoic acid was obtained from this soil was as follows:

The soil was treated at room temperature with a 2 per cent solution of sodium hydroxid for six hours, allowed to stand several hours, and the

colored extract siphoned off. This extract was acidified with sulphuric acid, filtered, and the acid filtrate shaken out several times with ether. The ether extracts were combined and the ether evaporated on the surface of a small quantity of warm water. The water was then heated to boiling and filtered hot, when, on cooling the filtrate, crystals separated. A further yield of crystals was obtained on concentrating the mother liquor from the crystals first obtained. The compound obtained in this way was purified by recrystallizing from water, and finally by sublimation, when a pure white product was obtained. About 2 grams were obtained from 25 kilograms of soil.

This compound had all the properties of benzoic acid. It crystallized in the leaflets characteristic of benzoic acid. It was readily soluble in alcohol, ether, and chloroform, sparingly soluble in cold water but much more readily in hot water, and melted at  $121^{\circ}\text{C}$ . An aqueous solution was acid in reaction, and when neutralized and treated with a neutral solution of ferric chlorid a dirty-brown precipitate was formed that was insoluble in acetic acid. The compound sublimed readily and when heated strongly gave off the irritating fumes characteristic of benzoic acid when so treated. Finally it gave Mohler's reaction.<sup>1</sup>

The appearance and properties of the compound obtained from the soil, its behavior with ferric chlorid, and response to Mohler's test are sufficient to establish its identity as benzoic acid.

#### METAOXYTOLUIC ACID

Metaoxytoluic acid was obtained from several samples of the series examined, but in quantity only from subsoils. The method by which it was obtained was exactly the same as that just outlined for benzoic acid up to the point of obtaining a water solution of the ether extract. If no benzoic acid or other compound separated on cooling the filtrate, it was concentrated nearly to dryness and allowed to stand, when oxytoluic acid, if present, crystallized out. The compound so obtained was purified by repeated recrystallizations from water and was finally dried on a porous plate. This product retained persistently a slight tinge of color, and it was only after many treatments and much loss of material that it could be freed from color. Where traces of benzoic acid accompanied it, as seemed to be the case in some instances, it could be freed from benzoic acid by sublimation, this acid being much more readily sublimed than oxytoluic acid. About 10 grams of pure material were obtained from 25 kilograms of soil.

<sup>1</sup> Mohler's test is carried out by heating the substance to be determined with sulphuric acid until charring takes place, sulphobenzoic acid being formed if benzoic acid is present in the original material. On treating with potassium nitrate this will be transformed into metadinitro-benzoic acid. On adding excess of ammonia to this acid and then a few drops of a colorless solution of ammonium sulphid a red color will be obtained.

Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 181, 1908.

Mohler, E. Recherche de l'acide benzoïque dans les substances alimentaires. Bul. Soc. Chim., Paris, s. 3, t. 3, p. 414-416, 1890.

On elementary analysis this compound gave the following results (0.200 gram were used for each analysis):

	Analysis 1. Per cent.	Analysis 2. Per cent.
Carbon.....	63.33	63.02
Hydrogen.....	5.39	5.66
Oxygen.....	31.28	31.32

This corresponds with the composition of oxytoluic acid,  $C_8H_8O_3$ , which contains 63.15 per cent of carbon, 5.26 per cent of hydrogen, and 31.50 per cent of oxygen. There are 10 isomeric oxytoluic acids, all of which have been described. The compound obtained from the soil has the properties of metaoxytoluic acid, with the carboxyl, hydroxyl, and methyl radicals in the 1. 3. 5. positions, respectively.

This compound crystallizes in plates or, when the quantity is small, in groups of radiating needles. It is rather soluble in cold water, but more so in hot water. It is soluble in alcohol and ether, sublimes unchanged, and melts at  $208^\circ C$ . On the addition of a solution of ferric chlorid, an aqueous solution of the compound gives a brown precipitate which dissolves to a brown solution when the reagent is added in excess. On the distillation of the dry compound with lime, metacresol is formed. The identity of the metacresol obtained from the soil compound in this way was established by transforming it into 2. 4. 6. trinitrocresol, a yellow compound melting at  $106^\circ$  to  $107^\circ C$ . This was effected by dissolving the metacresol in strong sulphuric acid, pouring into a mixture of nitric and sulphuric acids, heating, and then cooling. The nitro product was filtered off, washed with dilute hydrochloric acid, recrystallized, and dried.

Metaoxytoluic acid was made from sulphotoluic acid according to the method of Jacobsen,<sup>1</sup> and its properties were compared with the compound obtained from the soil, the two agreeing in every respect. When the artificial product and the soil compound were mixed, the melting point was unchanged. The agreement in composition and properties mentioned is sufficient to establish the identity of the compound obtained from the soil as metaoxytoluic acid.

#### VANILLIN

In the course of investigations of the organic matter of soils carried on for the past six years soil extracts having the odor of vanillin and giving some of the reactions of that compound have been encountered from time to time, but its presence could not be confirmed by isolation in pure form. In investigating the soil samples from Florida the isolation was accomplished.

The method of isolation, as with the compounds just described, was begun by making an alkaline extract of the soil. This extract was acidified and filtered and was then shaken out with several portions of ether.

<sup>1</sup> Jacobsen, Oscar. Oxytoluylsäuren und Oxyphthalensäuren. Ber. Deut. Chem. Gesell., Jahrg. 14, Juli-Dez., p. 2357-2359, 1881.

The combined ether extracts were shaken with a strong solution of sodium bisulphite, which treatment removes from the ether compounds of an aldehyde nature. After separating the bisulphite from the ether it was acidified with enough sulphuric acid to decompose all the bisulphite, was freed from sulphur dioxide by blowing air through it, and was again shaken with ether. On evaporating the ether extract at room temperature a more or less oily, viscous residue remained which had the odor of vanillin and gave the reactions of that compound. Crystals usually separated from this residue after standing several days.

When these crystals were obtained in sufficient quantity, they were purified by the method recommended for the examination of vanilla extracts.<sup>1</sup> The oily residue was treated several times with warm water and filtered, and the filtrate treated with a solution of lead acetate as long as a precipitate formed, and was then again filtered. The filtrate was shaken out several times with ether, and the combined ether extracts were evaporated. The residue usually crystallized readily, although it still contained traces of resinous matter. This resinous material could not be removed by taking up in ammonia, acidifying, and again shaking with ether as recommended in the method for vanilla extracts, but by recrystallizing from water several times and finally drying on a porous plate pure crystals were obtained.

These crystals had a strong odor of vanilla, were in the form of needles or small prisms, and melted at 80° to 81° C., the melting point of vanillin. They were readily soluble in ether or alcohol, but were sparingly soluble in water. An aqueous solution gave the following reactions characteristic of vanillin or the group of compounds to which vanillin belongs:

The addition of a solution of ferric chlorid gave a blue-violet color. Colors ranging from blue to violet are given by many hydroxy-benzene compounds.

When boiled with resorcinol and hydrochloric acid, a red color was formed. This reaction is given by a number of aldehydes, including some sugars.

When the crystals were treated with equal quantities of sulphuric and hydrochloric acids and with the addition of a drop of a dilute solution of acetone and the mixture then heated to 100° C. for 15 minutes, a violet color was developed.

On adding an excess of bromin water followed by the addition of ferrous sulphate, a blue-green color was formed. This reaction is regarded as characteristic of vanillin, but does not seem applicable for colorimetric determination.

When the reagent of Folin and Denis<sup>2</sup> was added and the mixture made alkaline with an excess of sodium carbonate, a clear blue color was

<sup>1</sup> Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 156, 1908.

<sup>2</sup> Folin, Otto, and Denis, W. On phosphotungstic-phosphomolybdic compounds as color reagents. Jour. Biol. Chem., v. 12, no. 2, pp. 239-243, 1912.



slowly developed. This reaches a maximum in a short time and remains constant for several hours, furnishing a reliable colorimetric method for the determination of vanillin.<sup>1</sup>

The method of isolating the compound from the soil, its crystalline form, odor, melting point, and the fact that it gave the characteristic reactions of vanillin are sufficient to establish its identity as vanillin.

The quantity of pure vanillin obtained by this method from any of the soils examined was but a few milligrams from 25 kilograms. It was possible to obtain the vanillin in pure crystalline form from 4 of the 16 samples. From some of the other samples crystals were obtained that gave the reactions of vanillin, but there were not enough for the separation and determination of the melting point. Residues were obtained from all the samples having the odor of vanillin and giving two or more reactions for it. In each case where it was possible to separate vanillin in pure form the sample was a surface soil.

An application of the colorimetric method of Folin and Denis was made to two samples, those from which the most vanillin had been obtained by alkaline extraction. One hundred grams of soil were finely ground and thoroughly extracted with warm alcohol that had been freshly distilled. The alcohol was evaporated, and the residue was taken up with warm water and filtered. Lead acetate was added to the filtrate as long as a precipitate formed. The solution was then filtered and the filtrate treated with the reagent of Folin and Denis (a mixture of phosphotungstic and phosphomolybdic acids), followed by an excess of sodium carbonate. It was again filtered and made to a definite volume. The resulting blue solution was read in a colorimeter against a solution prepared in the same way from a standard solution of vanillin. Sample No. 1 gave 0.0010 per cent of vanillin, or 10 parts per million, while sample No. 2 showed 0.00048 per cent, or 4.8 parts per million.

Vanillin contains the radical methoxyl  $\text{OCH}_3$ . In a previous paper<sup>2</sup> it was shown that the methoxyl radical is present in many soils in sufficient quantity to be determined by the Zeisel method.<sup>3</sup> A determination of the methoxyl in samples Nos. 1 and 2 by this method gave, for sample No. 1, 0.065 per cent of methoxyl calculated to vanillin, and for sample No. 2, 0.050 per cent.

Methoxyl is contained in a number of organic compounds and is a constant constituent of the lignocellulose of plants. The quantity obtained from these soils when calculated to vanillin is so much in excess of that actually obtained in the isolation from an alkaline extract, or that

<sup>1</sup> Folin, Otto, and Denis, W. A new colorimetric method for the determination of vanillin in flavoring extracts. *Jour. Indus. and Engin. Chem.*, v. 4, no. 9, pp. 670-672, 1912.

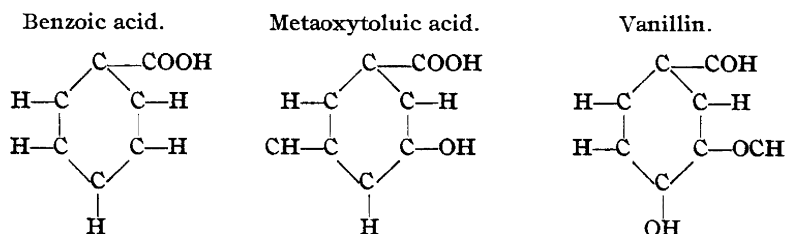
<sup>2</sup> Shorey, E. C., and Lathrop, E. C. Methoxyl in soil organic matter. *Jour. Amer. Chem. Soc.*, v. 33, no. 1, p. 75-78, 1911.

<sup>3</sup> Zeisel, S. Über ein Verfahren zum quantitativen Nachweise von Methoxyl. *Monatsh. Chem.*, Bd. 6, 1885, p. 989-996, 1 pl. 1886.

— Zum quantitativen Nachweise von Methoxyl. *Monatsh. Chem.*, Bd. 7, 1886, p. 406, 409. 1887.

indicated by the Folin-Denis method, that it seems evident that a considerable portion of it must be derived from compounds other than vanillin.

These three compounds, benzoic acid, metaoxytoluic acid, and vanillin, although not related in the sense that they are readily derived from or transformed into one another, are related as shown by the following structural formulas:



Benzoic acid is a naturally occurring product obtained from certain gums and balsams, wherein it exists as an ester. It is also present in some fruits, such as plums and cranberries, and has been found among the oxidation products of casein and gelatin. Its presence in soil might then result from the decay of plant tissues containing it or from oxidation of more complex compounds through the activity of microorganisms. The most remarkable fact in connection with its occurrence in the soils examined is that it was found in appreciable quantity in but one sample, although they were of the same general character. In the absence of accurate information regarding previous natural vegetation on these soils and other data that can be obtained only in the field, any attempt to explain this fact is out of place here.

Metaoxytoluic acid, so far as known, is not a natural product, and its method of preparation in the laboratory does not suggest any process by which it might be formed in the soil from plant products or other compounds known to occur in soils.

Vanillin has its chief natural source in the so-called vanilla beans, or seed pods, of *Vanilla pompona*. It has also been reported as found in small quantities in a number of other plants or plant products, and it probably is more widely distributed in the vegetable kingdom than has been supposed. At present there is no information indicating its formation from other compounds through the agency of microorganisms, and the small quantity found in soils may possibly be regarded as an unchanged residue of plant debris.

Using the maximum figures for quantities obtained in these investigations and calculating to the acre-foot of soil, the following approximate quantities are obtained: Benzoic acid, 350 pounds; metaoxytoluic acid, 800 pounds; and vanillin, 40 pounds to the acre-foot.

In the case of the two acids the method involved considerable loss of material and the actual quantity present in the soil is undoubtedly in excess of these figures.

The question as to the form in which these compounds exist in the soil is one deserving some consideration, although one not easily answered satisfactorily. It is true of most organic compounds that have been obtained from soils through extraction with dilute alkali that they are not readily obtained as such by water extraction of the soil. In many soils this can be explained, in part at least, by the fact that much of the organic matter in soils is of a resinous nature wholly insoluble in water, and compounds which when separated are easily soluble in water are so incased or protected by the resinous or varnishlike coating effected by this resinous material that they are very slowly dissolved, if at all, when the soil is leached. This effect is quite apart from any absorptive effect and is quite marked in extreme types, such as the sands of Florida and some peats, where either fine grinding or previous treatment with alcohol will render soluble in water organic material that before this treatment was so little soluble as to escape notice.

In the case of vanillin, grinding the soil and extracting with alcohol gave more of the compound than was obtained by extraction with alkali, and from the known properties of vanillin it seems unlikely that the quantity found is in the soil in any other form than free vanillin.

Treatment of the soil with hot alcohol after grinding gave extracts from which reactions for both benzoic acid and metaxytoluic acid could be obtained, but in the absence of colorimetric methods applicable to small quantities and owing to the fact that the extractions with alcohol were made with much smaller quantities of soil than the extraction with sodium hydroxid, no comparative figures can be given. It is fair to conclude, however, that in some of the soils examined some portion of both acids is present as free acid.



# INDICATOR SIGNIFICANCE OF VEGETATION IN TOOELE VALLEY, UTAH

By T. H. KEARNEY,<sup>1</sup> L. J. BRIGGS,<sup>2</sup> H. L. SHANTZ,<sup>3</sup> J. W. McLANE,<sup>4</sup> and  
R. L. PIEMEISEL,<sup>5</sup>

*Bureau of Plant Industry*

## INTRODUCTION

In the arid portion of the United States the different types of native vegetation are often very sharply delimited, the transitions being so abrupt that they can not be attributed to climatic factors; this has suggested the possibility of correlating the distribution of the vegetation with the physical and chemical properties of the soil. If such correlations can be made, they may be utilized in the classification of land with respect to its agricultural capabilities.

One of the writers<sup>6</sup> has described the correlations which exist in the Great Plains between the different types of vegetation and the physical characteristics of the corresponding types of land and has pointed out how the native growth may be used in that region to determine the suitability of the land for dry farming.

The results obtained in the Great Plains made it desirable to undertake similar investigations in the Great Basin region, or that portion of the United States lying between the Rocky Mountains on the east and the Sierra Nevada and Cascade Ranges on the west. The problems to be solved were: First, what types of vegetation indicate conditions of soil moisture favorable or unfavorable to dry farming, and, second, what types indicate the presence or absence of alkali salts in quantities likely to injure cultivated crops. For the purpose of this investigation it was necessary to find a locality where both dry farming and irrigation farming are practiced, where much of the land is still covered with the original native growth, and where some of the soils contain an excess of alkali salts.

---

<sup>1</sup> Physiologist in Charge, Alkali and Drought Resistant Plant Investigations.

<sup>2</sup> Biophysicist in Charge, Biophysical Investigations.

<sup>3</sup> Plant Physiologist, Alkali and Drought Resistant Plant Investigations.

<sup>4</sup> Laboratory Assistant, Biophysical Investigations.

<sup>5</sup> Scientific Assistant, Alkali and Drought Resistant Plant Investigations.

<sup>6</sup> Shantz, H. L. Natural vegetation as an indicator of the capabilities of land for crop production in the Great Plains area. U. S. Dept. Agr., Bur. Plant Indus. Bul. 201, 100 p., 23 fig., 6 pl. 1911.

With the exception of the valuable work of Hilgard in Mississippi and of Hilgard and his associates in California (see Hilgard, E. W., Soils, New York, 1906, p. 487-548, figs. 77-89), very little had previously been done in the United States toward a scientific study of native vegetation from the indicator point of view. In Europe, however, the subject has been much investigated, especially as regards "lime-loving" and "lime-avoiding" plants.

After a reconnoissance trip through portions of Wyoming, Utah, Idaho, and Oregon in August, 1911, the Tooele Valley in central Utah was selected for the following reasons: (1) several very distinct types of vegetation are found within a small area, (2) the soils show a great diversity in their moisture conditions and salt content, (3) the greater part of the area retains its original plant cover, while examples of crop production both with and without irrigation exist on different types of land.

Detailed studies of the vegetation of Tooele Valley in relation to the moisture conditions and salt content of the soil were carried on in 1912. The work was begun near the close of the rainy season (end of May) and was terminated during the first week of August, when the summer drought had reached its height. Additional data were obtained during a third visit to the valley in the latter part of August, 1913.

The distribution of the native vegetation was found to depend in a marked degree upon the physical and chemical properties of the soils, factors which also influence crop production. So far as this particular area is concerned, the vegetation can unquestionably be used with advantage in classifying land with respect to its agricultural value. To what extent the correlations established in Tooele Valley hold good in other parts of the Great Basin region remains to be determined by future investigation.

The writers desire to acknowledge the helpful cooperation of Director E. D. Ball, of the Utah Agricultural Experiment Station, and of Prof. L. A. Merrill, formerly of that station. The writers are indebted for the determination of the plants collected to Mr. Ivar Tidestrom, of the Office of Economic and Systematic Botany, Bureau of Plant Industry.

#### METHODS OF RESEARCH

The methods used in classifying and describing the types of vegetation are well known to ecological plant geographers and are best described in setting forth the results. Some explanation of the methods used in investigating the moisture conditions and salinity of the soils, however, is desirable.

Samples of the soil were taken in the midst of the areas occupied by each vegetation type. Where the boundaries between two types were well defined, samples were also taken on both sides of the line, in order to determine the limiting conditions for each type. The measurements of moisture content, moisture equivalent, electrical resistance, and salt content which were made upon these samples served as a basis for conclusions regarding the physical conditions indicated by the presence of each important type of vegetation.

COLLECTING SOIL SAMPLES.—The samples of soil were in all cases collected with the aid of a sampling tube, which prevents the admixture of surface material with the subsoil. Each sample consisted of a

composite of four cores. The soils were usually sampled to a depth of 4 feet and occasionally to a greater depth, the cores being taken in 1-foot sections.

**DETERMINING THE SOIL-MOISTURE CONTENT.**—Numbered tin boxes of uniform weight and with tight-fitting covers were used to receive the soil samples directly from the sampling tubes. The whole sample was used as a basis for the moisture determinations and after the initial weighing was dried in a water oven to constant weight. The moisture content is in all cases expressed as a percentage of the dry weight of the sample.

**DETERMINING THE MOISTURE EQUIVALENT.**—In studying soil moisture in relation to plant growth it is important to have some standard for measurement of the retentivity of the soil for moisture. As two of the authors have previously shown,<sup>1</sup> this may be conveniently accomplished by the method of moisture equivalents. This method consists in subjecting a moist sample of soil to a constant centrifugal force equal to 1,000 times that of gravity until the moisture content of the soil is reduced to the point where it is in equilibrium with the centrifugal force employed. The residual moisture content of the soil is then determined. This value, expressed as a percentage of the dry weight of the sample, is the moisture equivalent. A direct measure of the retentiveness for moisture of the various soils is thus obtained, and, since the same force is employed throughout, all of the determinations are directly comparable.

**DETERMINING THE WILTING COEFFICIENT.**—It has been shown by two of the authors<sup>2</sup> that the moisture equivalent serves as a useful indirect means of determining the wilting coefficient. The latter term designates (as a percentage of the dry weight of the soil) the quantity of water remaining in the volume of soil occupied by the active roots of a plant which is beginning to wilt.<sup>3</sup>

These determinations (moisture equivalent and wilting coefficient) serve to give an idea of the texture of the soils occupied by the different plant associations, as indicated by their retentiveness for moisture. By subtracting the wilting coefficient from the actual moisture content a measure is obtained of the percentage of moisture available for the active growth of plants at the time the soil samples were taken.

**DETERMINING THE SALT CONTENT.**—The total salt content of each soil sample was determined by the electrical-resistance method developed

<sup>1</sup> Briggs, L. J., and McLane, J. W. Moisture equivalents of soils. U. S. Dept. Agr., Bur. Soils Bul. 45, 23 p., 1 fig., 1 pl. 1907.

Briggs, L. J., and McLane, J. W. Moisture-equivalent determinations and their application. Proc. Amer. Soc. Agron., v. 2, 1910, p. 138-147, pl. 6. 1912.

<sup>2</sup> Briggs, L. J., and Shantz, H. L. The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agr., Bur. Plant Indus. Bul. 230, 83 p., 9 fig., 2 pl., 1912.

<sup>3</sup> "Wilting" in this case must be understood as permanent wilting—i. e., a condition from which the plant can not recover its turgidity until the soil receives additional moisture, no matter how great the humidity of the atmosphere.

in the Bureau of Soils.<sup>1</sup> The method is simple and rapid and the measurements can be readily made in the field, which is a great advantage in studying the distribution of vegetation in relation to the salt content of the soil. The method is, however, necessarily an approximate one, owing to the variation in the composition of the soil solution and to the fact that the salts found in soils differ greatly with respect to their molecular weight and ionic migration velocity. To interpret the observed resistance, a calibration curve was prepared, based upon the observed relationship between the electrical resistance and the salt content, gravimetrically determined, of a number of soils from different parts of the valley. (See fig. 1.)

In making the gravimetric determinations, the usual practice was followed of digesting 100 grams of dry soil with 500 c. c. of water, filtering, and evaporating an aliquot

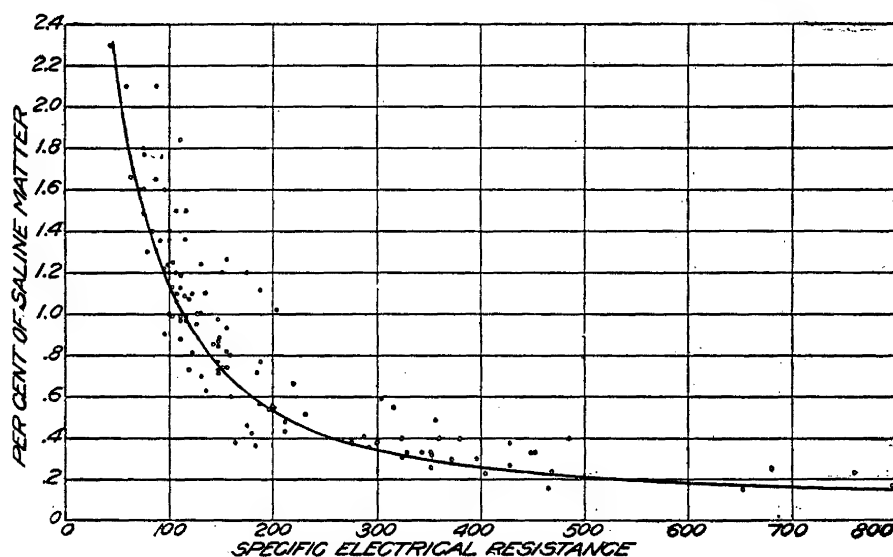


FIG. 1.—Curve showing the relation between the salt content (in percentages of the dry weight of the soil) and the specific electrical resistance (in ohms) of the soil when saturated with water.

portion of the filtrate to dryness. A number of the samples examined were rich in gypsum, and in digesting such soils with an excess of water the total quantity of gypsum which goes into solution is greatly in excess of the quantity dissolved when the soil is simply saturated with water. The gravimetric determination of the salt content of soils which are rich in gypsum is consequently too high, and this accounts in part at least for the outlying points above the calibration curve. (Fig. 1.)

By means of a suitable centrifugal apparatus it is possible to remove and collect a portion of the soil solution in an unsaturated soil. From the concentration of this solution and the initial moisture content of the soil, the salt content of the soil can be calculated. This method gave results more nearly in accord with those indicated by

<sup>1</sup> Whitney, Milton, and Means, T. H. An electrical method of determining the soluble salt content of soils. U. S. Dept. Agr., Div. Soils Bul. 8, 30 p., 6 fig. 1897.

Briggs, L. J. Electrical instruments for determining the moisture, temperature, and soluble salt content of soils. U. S. Dept. Agr., Div. Soils Bul. 15, 35 p., 12 fig. 1899.

Davis, R. O. E., and Bryan, H. The electrical bridge for the determination of soluble salts in soils. U. S. Dept. Agr., Bur. Soils Bul. 61, 36 p., 7 fig. 2 pl. 1910.



the electrical resistance. Therefore, in the case of soils containing gypsum the electrical-resistance method may be considered to be more reliable than the excess-solvent method. The probable error of determinations by the electrical-resistance method is approximately 10 per cent of the actual salt content.

#### CLIMATE OF TOOEELE VALLEY

Tooele Valley is dry, having a mean annual precipitation of 16 inches.<sup>1</sup> The average monthly distribution of the precipitation at Tooele is shown in figure 2. No precipitation records are available for other parts of the valley, save fragmentary records at Grantsville for two years, which indicate that the western side of the valley receives decidedly less precipitation than the eastern slope. During the first nine months of 1912, the total precipitation recorded at Grantsville was 7.6 inches, as compared with 13 inches at Tooele. The condition of the native vegetation and of the crops grown without irrigation also indicates that the western side of the valley is much drier than the eastern side.

In view of the importance of the soil-moisture conditions in explaining the distribution of the different types of vegetation in Tooele Valley, it is interesting to consider

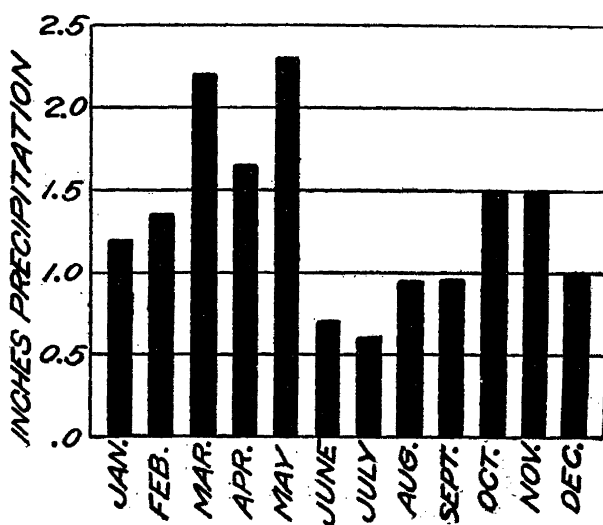


FIG. 2.—Monthly distribution of precipitation at Tooele, Utah (mean for 15 years).

the precipitation of the period immediately prior to that during which the field work was carried on. The precipitation during the months from October to May, inclusive, probably furnishes all of the stored soil moisture available for the growth of plants during the following summer. The total precipitation at Tooele during the period from October, 1911, to May, 1912, was 13.5 inches, or 0.9 inch above the normal (12.6 inches) for the locality. Hence, it may be assumed that at least the normal quantity of moisture was present in the soil on the date when field operations were begun in the valley (May 28). As regards the season of active growth in 1912, the precipitation of the month of May was about 0.5 inch below the normal for Tooele, while that of June was very nearly twice the normal. For the remaining summer months the precipitation was about normal.

<sup>1</sup> Based upon 15 years' measurements at the town of Tooele. From data furnished by the U. S. Weather Bureau, through the courtesy of Mr. A. H. Thiessen, Section Director.

While no evaporation data are available for Tooele Valley, evaporation measurements<sup>1</sup> have been made during the last five years at Nephi, about 60 miles south of Tooele. These measurements show that the monthly evaporation during June, July, and August is at least double that of April and October. (See Table I.)

TABLE I.—*Evaporation from a free-water surface at Nephi, Utah, during the months of April to October, 1908 to 1912.*

Year.	April.	May.	June.	July.	August.	Sep-tember.	October.
	<i>Inches.</i>	<i>Inches.</i>	<i>Inches.</i>	<i>Inches.</i>	<i>Inches.</i>	<i>Inches.</i>	<i>Inches.</i>
1908.....			7.87	10.52	9.34	6.23	.....
1909.....	3.64	5.99	8.81	9.47	7.03	5.59	4.43
1910.....	5.82	7.46	10.90	9.98	10.09	6.01	3.72
1911.....	4.93	8.41	8.69	8.72	10.47	6.69	3.65
1912.....	3.54	6.30	9.28	9.24	8.89	6.16	2.98
Normal.....	4.48	7.04	9.11	9.59	9.16	6.14	3.70

Therefore, while the summer months are by no means rainless in this locality, the great increase in the rate of evaporation is such that the light precipitation can have but little effect upon vegetation. In those parts of the valley where the ground water is beyond the reach of the plant roots the vegetation becomes dormant after the moisture stored in the soil by the winter and spring rains has been exhausted. Herbaceous plants ripen and die, at least to the ground, while the woody species, losing much of their foliage and reducing their transpiration to a minimum, enter a resting condition which is nearly as complete as that which is brought about by the low temperatures of winter. Where there is a greater depth of readily permeable soil in which moisture can be stored than is ordinarily the case in this valley, the beginning of summer dormancy is longer postponed. On the sand hills the larger shrubs may continue growing more or less actively throughout the summer. In the lower part of the valley, where the ground-water table is high and the soil is moist throughout the summer nearly or quite to the surface, active growth continues until it is terminated by frosts.

#### GEOLOGY AND TOPOGRAPHY OF TOOEE VALLEY

Geologically, Tooele Valley is of exceptional interest because of its occupancy at one time by a bay of Lake Bonneville, a Pleistocene lake, the beach lines of which are strikingly in evidence upon the sides of the surrounding mountains. The highest of these terraces is 1,000 feet above the present surface of Great Salt Lake. An exhaustive study of the region has been made by Gilbert.<sup>2</sup>

<sup>1</sup> Measurements by the Office of Biophysical Investigations in cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, and with the Utah Agricultural Experiment Station.

<sup>2</sup> Gilbert, G. K. *Lake Bonneville*. 438 p., 51 illus., 51 pls. Washington. 1890. (U. S. Geol. Survey Monograph 1.)

Tooele Valley is broadly U-shaped in cross section, the mountains on either side rising somewhat abruptly from the valley floor. This abrupt change from valley plain to mountain is characteristic of many of the valleys of the region and is due to the extensive deposition of alluvium during some epoch prior to the Bonneville period.

Tooele Valley is bounded on the east by the Oquirrh Mountains and on the west by the Stansbury or Aquí Range. The southern boundary is formed by a spur of the Stansbury Range and by the great Stockton embankment, which is composed of sand and water-worn gravel thrown up by the waters of Lake Bonneville and which separates Tooele Valley from Rush Valley. (The Stockton embankment is shown in extreme background of Pl. XLV, fig. 3.) The summit of this embankment coincides with the highest shore line on the adjacent mountains. To the north the valley slopes downward to the southern shore of Great Salt Lake. The axis of the valley thus lies approximately on a north and south line, the land rising gradually from near the center to the mountain ranges on the east and west sides. The width of Tooele Valley at the northern end is about 18 miles, at the southern end it is about 13 miles, and its greatest length is approximately 16 miles. The total area of the valley floor is, roughly, 250 square miles.

The slope of the valley from the sides and from the southern end to a line marked approximately by the highway from Salt Lake City to Grantsville is decidedly steep, as is indicated by the fact that the town of Tooele has an elevation above sea level of 4,900 feet, while Grantsville, although less than 5 miles farther north, is 680 feet lower.<sup>1</sup> North of this line the slope becomes very gentle and the surface of this portion of the valley is plainlike.

#### SALINE CONDITIONS OF TOOËLE VALLEY

The soils of Tooele Valley show a wide range in salinity, or, to use the more familiar term, in "alkali" content. The soils in the upper end of the valley and along the base of the foothills at either side, including a large alluvial fan northeast of the town of Tooele, are characterized by a low salt content. The other extreme is found in the flats adjacent to the lake, which in some cases contain such an excess of soluble salts as to prevent the development of a plant cover. The soils occupying the central portion of the valley are, as a rule, relatively free from salts in the surface foot, but the salinity of the subsoil is usually such as to exclude all deep-rooted plants except those that are salt-tolerant to a marked degree. The saline material in solution in the nearly saturated soils of the flats, like that of the lake itself, is made up largely of sodium chlorid. In fact, these flats have probably not infrequently been submerged by the rise of the lake, since records made by the United States

<sup>1</sup> The elevation of the surface of the water of Great Salt Lake is about 4,200 feet.

Geological Survey show that within the last 40 years the lake has undergone a fluctuation in level of 16 feet.

The following determinations of the composition of the saline material in Great Salt Lake, which are quoted from a compilation by Clarke,<sup>1</sup> are therefore of interest in showing what may be regarded as the typical composition of the saline material in this part of the area.

TABLE II.—Analyses of water from Great Salt Lake.<sup>1</sup>

	A	B	C	D	E	F	G	H
Cl.....	55.99	56.21	55.57	56.54	55.69	55.25	55.11	53.72
Br.....	Trace.				Trace.	Trace.		
SO <sub>4</sub> .....	6.57	6.89	6.86	5.97	6.52	6.73	6.66	5.95
CO <sub>3</sub> .....		.07						
Li.....	Trace.				.01	Trace.		
Na.....	33.15	33.45	33.17	33.39	32.92	34.65	32.97	32.81
K.....	1.60	(?)	1.59	1.08	1.70	2.64	3.13	4.99
Ca.....	.17	.20	.21	.42	1.05	.16	.17	.31
Mg.....	2.52	3.18	2.60	2.60	2.10	.57	1.96	2.22
(Fe <sub>2</sub> O <sub>3</sub> , Al <sub>2</sub> O <sub>3</sub> , SiO <sub>2</sub> ).....					.01			
Salinity, per cent.....	100.00 14.994	100.00 13.790	100.00 15.671	100.00 19.558	100.00 123.036	100.00 27.72	100.00 22.99	100.00 17.68

<sup>1</sup> More correctly 230.355 grams per liter.

"A. By O. D. Allen, Rept. U. S. Geol. Expl. 40th Par., vol. 2, 1877, p. 433. Water collected in 1869. A trace of boric acid is also reported, in addition to the substances named in the table. Allen also gives analyses of a saline soil from a mud flat near Great Salt Lake. It contained 16.40 per cent of soluble matter much like that of the lake water.

"B. By Charles Smart. Cited in Resources and attractions of the Territory of Utah, Omaha, 1879. Analysis made in 1877.

"C. By E. von Cochenhausen, for C. Ochsenius, Zeitschr. Deutsch. geol. Gesell., vol. 34, 1882, p. 359. Sample collected by Ochsenius April 16, 1879. Ochsenius also gives an analysis of the salt manufactured from the water of Great Salt Lake.

"D. By J. E. Talmage, Science, vol. 14, 1889, p. 445. Collected in 1889. An analysis of a sample taken in 1885 is also given.

"E. By E. Waller. School of Mines Quart., vol. 14, 1892, p. 57. A trace of boric acid is also reported.

"F. By W. Blum. Collected in 1904. Recalculated to 100 per cent. Reported by Talmage in Scottish Geog. Mag., vol. 20, 1904, p. 424. An earlier paper by Talmage on the lake is in the same journal, vol. 17, 1901, p. 617.

"G. By W. C. Ebaugh and K. Williams, Chem. Zeitung, vol. 32, 1908, p. 409. Collected in October, 1907.

"H. By W. Macfarlane, Science, vol. 32, 1910, p. 568. Collected in February, 1910. A number of other analyses, complete or incomplete, are cited in this paper by Ebaugh and Macfarlane."

It will appear from these analyses that sodium and chlorin together constitute about 90 per cent of the total soluble material. The quantity of chlorin is, in each analysis, slightly greater than that necessary to satisfy the basic requirements of sodium. The rest of the soluble material is made up almost wholly of potassium, magnesium, and the sulphate radical. Concerning these analyses Clarke<sup>2</sup> says:

Although the salinity of the lake is very variable and from four to seven times as great as that of the ocean, its saline matter has nearly the same composition. The

<sup>1</sup> Clarke, F. W. Data of geochemistry. U. S. Geol. Survey Bul. 491, ed. 2, p. 144. 1911.

<sup>2</sup> Clarke, F. W. Op. cit.

absence of carbonates, the higher sodium, and the lower magnesium are the most definite variations from the oceanic standard; but the general similarity, the identity of type, is unmistakable. \* \* \*

All the waters tributary to Great Salt Lake, so far as they have been examined, contain notable quantities of carbonates, which are absent from the lake itself. These salts have evidently been precipitated from solution, and evidence of this process is found in beds of oolitic sand, composed mainly of calcium carbonate, which exist at various points along the lake shore. The strong brine of the lake seems to be incapable of holding calcium carbonate in solution.

The analyses as given in Table II report the presence of carbonates in solution in the lake water in only one instance.<sup>1</sup> It is in this respect that the saline matter of the soils more distant from the lake differs most markedly from the type just considered. Calcium carbonate was found widely distributed in the soils of the valley. Sodium carbonate was often found also, usually in small amounts (0.05 to 0.10 per cent of the dry weight of the soil), but occasionally samples were collected containing as high as 0.25 per cent. Sodium carbonate was found most frequently in the samples collected in areas where *Kochia* was growing. These soils were also highly calcareous. The available data on the distribution of sodium carbonate do not, however, indicate that it can be correlated with the presence of any particular plant community.

The composition of the salts of Great Salt Lake would lead one to expect that the chlorids would prove to be the most common and widely distributed of the saline constituents of the Tooele Valley soils, and such has been found to be the case. In the course of the work a quantitative examination for chlorids was made of 162 samples of soil, and all but 13 samples showed the presence of measurable quantities of chlorids. Of these 13 exceptions 12 were samples from *Artemisia tridentata* (sagebrush) areas which are characterized by a very low total salt content. The sodium-chlorid content of the areas examined, all of which were occupied by vegetation of one type or another, ranged from a trace in the land occupied by *Artemisia* to over 2 per cent in land occupied by *Allenrolfea occidentalis*. Outside of the sagebrush areas the sodium-chlorid content of most of the samples fell between 0.4 and 1.3 per cent. In a large majority of the samples examined sodium chlorid constituted more than one-half of the total water-soluble material.

Sulphates are usually present in the soils containing an excess of salts. Of 122 samples examined 96 showed the presence of sulphates. It is well recognized through the researches of Hilgard and others that calcium sulphate is a corrective for the soluble "black alkali" (sodium carbonate), the reaction between these salts resulting in the formation of the

<sup>1</sup> F. K. Cameron has shown, however, that while the lake water at its normal concentration does not give an alkaline reaction with phenolphthalein, this reaction will develop simply by diluting the lake water with distilled water. At the normal concentration of the lake, the dissociation of the sodium carbonate is held back through the great number of sodium ions resulting from the dissociation of the sodium chlorid. The lake does, therefore, carry a slight amount of sodium carbonate. (Gardner, F. D., and Stewart, John. A soil survey in Salt Lake Valley, Utah. U. S. Dept. Agr., Div. Soils Field Operations, Rpt. 64, 1899, p. 104-105. 1900.)

relatively insoluble calcium carbonate and neutral sodium sulphate. It is evident that a similar reaction would take place if magnesium sulphate were present, since magnesium also forms an insoluble carbonate. It consequently seemed desirable to examine the carbonate and sulphate measurements with a view to determining to what extent the absence of soluble carbonates was accompanied by the presence of sulphates. Of 122 samples examined for carbonates and sulphates 13 contained neither carbonates nor sulphates, while 13 others contained carbonates but no sulphates, leaving 96 samples containing sulphates. Of these, 78 samples were free from carbonates, 2 samples contained both carbonates and sulphates in measurable quantities, while in the remaining 16 samples traces only of both sulphates and carbonates were present.

#### VEGETATION OF TOOELE VALLEY

The plant covering of the area under consideration is typical of a large portion of the Great Basin, several of the most important types of vegetation of that region being represented in Tooele Valley. Striking features of this vegetation are (1) the great extent of the areas occupied continuously by a single type of vegetation, (2) the sharpness of the boundaries between the areas occupied by each type, and (3) the great predominance of one or very few species in each type.<sup>1</sup>

#### CLASSIFICATION OF THE TYPES OF VEGETATION <sup>2</sup>

The principal types of vegetation of Tooele Valley, with the names of the species which are dominant in each, are listed in Table III.

---

<sup>1</sup> These are common characteristics of the vegetation of arid regions. Thus, Borszczow, as quoted by Ove Paulsen (*Studies on the Vegetation of the Transcaspiian Lowlands*. Copenhagen, 1912, p. 22-23), states:

"Here, as throughout the whole of Aralo-Caspia, it is a few specially characteristic forms which prevail; they repeat themselves continually so that the country has a very monotonous appearance. Other species are only subordinate to these. Where the character of the soil changes, these predominant species sometimes change very quickly and give place to others, which in turn prevail until the soil changes again. This monotony and this repetition of certain species over vast areas is the third characteristic of the vegetation of the Aralo-Caspian countries. It is no doubt a direct consequence of the uniformity of the climate, which again is mainly dependent on the slight vertical relief of the surface. \* \* \*

"In the Aralo-Caspian lands the soil in particular has such a great influence on the vegetation that a change of soil—other conditions remaining the same—often alters the physiognomy totally and almost abruptly without any gradual transitions."

<sup>2</sup> In view of the fact that the ecological plant geography of the Great Basin region is as yet but little understood, it seems inadvisable at this time to attempt to refer the plant associations of this valley to formations.

The term "plant association," as used in this paper, signifies an assemblage of plants occupying a relatively uniform environment, having an easily recognizable appearance or "physiognomy" and characterized by the predominance of one or few species.

TABLE III.—Types of the vegetation in Tooele Valley, Utah, and their dominant species.

Name of association or other plant community. <sup>1</sup>	Dominant species.
Sagebrush association.....	<i>Artemisia tridentata</i> .
Sand-hill mixed association.....	{ <i>Artemisia tridentata</i> . <i>Juniperus utahensis</i> . <i>Chrysothamnus nauseosus albicaulis</i> .
Kochia association.....	<i>Kochia vestita</i> .
Shadscale association.....	<i>Atriplex confertifolia</i> .
Greasewood-shadscale association.....	{ <i>Sarcobatus vermiculatus</i> . <i>Atriplex confertifolia</i> .
Grass-flat communities.....	{ <i>Distichlis spicata</i> . <i>Sporobolus airoides</i> . <i>Chrysothamnus graveolens glabrata</i> .
Salt-flat communities.....	{ <i>Allenrolfea occidentalis</i> . <i>Salicornia utahensis</i> . <i>Salicornia rubra</i> .

<sup>1</sup> Further investigation of the vegetation of the Great Basin region is needed before definite ecological rank can be assigned to the grass-flat and the salt-flat communities.

#### DISTRIBUTION OF THE TYPES OF VEGETATION

The distribution and relative area in Tooele Valley of the different types of vegetation is shown on the map (Pl. XLII).

Nearly all of the dry land free from alkali salts which retains the original plant covering is occupied by the sagebrush association. (Pl. XLIV.) This type of vegetation covers the southern end of the valley and also extends northward in a narrow fringe along the base of the Stansbury Range to within about 5 miles of Great Salt Lake. Few vestiges of the original cover remain on the eastern side of the valley, but there can be little doubt that sagebrush formerly occupied the bench lands and alluvial fans at the foot of the Oquirrh Range. The dominant species of this association is also found along gullies and in depressions, in the midst of areas otherwise occupied by the Kochia and shadscale associations. It is probable that most of the land now occupied by the sagebrush association was laid bare before the waters of Lake Bonneville had become strongly saline.

South of the center of the valley a rather extensive area of sand hills is covered by what may be designated the sand-hill mixed association. In this association also sagebrush is the dominant plant, but there is a plentiful admixture of Utah juniper and certain species of rabbit brush (*Chrysothamnus*), together with many herbaceous plants more or less peculiar to sandy soils. Botanically, this is the most varied and interesting type of vegetation occurring in Tooele Valley.

The middle portion of the valley resembles the upper portion in the dryness of the soil and subsoil during the summer, but differs in the high salt content of the subsoil. This territory is divided between two types of vegetation, the *Kochia* (Pl. XLVI) and the shadscale (Pl. XLVII, fig. 1) associations. The former occupies a sharply defined interrupted belt extending well across the valley just south of the sagebrush area and also penetrates the latter in the form of tongues and islands, which occur here and there far toward the head of the valley. (Pl. XLIII, fig. 2.) Lying just below the main *Kochia* belt an extensive tract is occupied by the shadscale association, which on the western side of the valley is prolonged in a gradually narrowing strip to the north end of the Stansbury Range. While the boundary between the sagebrush and *Kochia* associations is often very sharp (Pl. XLVI, fig. 1), that between the *Kochia* and shadscale associations is much less distinct. It is probable that the water of Lake Bonneville had become strongly saline before the areas now occupied by the *Kochia* and shadscale associations were laid bare and that the subsequent precipitation has been too small to leach the salts then deposited to a greater depth than 1 or 2 feet.

As the elevation of the land diminishes, the pure shadscale is gradually replaced by an association of greasewood and shadscale. The frontier between the two associations is not sharply defined (Pl. XLVII, fig. 2), scattered greasewood plants appearing first along gullies or draws and gradually, as Great Salt Lake is approached, mingling everywhere with the shadscale. This association extends to the edge of the lake, covering the summits of the low ridges and hummocks which are interspersed among the salt flats. In Tooele Valley greasewood scarcely occurs in a pure association, but is practically everywhere mingled with shadscale.

Between the main greasewood-shadscale area and the salt flats occur the grass flats, a nearly level expanse, marshy in places, covered largely with grasses and with a species of *Chrysothamnus*. (Pl. XLVIII, fig. 3.)

Near the present margin of the lake basinlike areas are found, many of which are doubtless under water at times. (Pl. XLIII, fig. 1; Pl. XLVIII, fig. 1.) The larger of these appear in summer as bare expanses covered with a glistening crust of white salts. Near their margins, however, and often covering the entire surface of the smaller depressions certain very salt-resistant plants occur, either scattered over the otherwise bare ground or forming rather dense colonies. The most important of these plants are *Allenrolfea occidentalis* (Pl. XLVIII, fig. 1), which is most at home on the slightly higher margins of the basins, and two species of glasswort (*Salicornia*)—one perennial (*S. utahensis*) (Pl. XLVIII, fig. 2), the other annual (*S. rubra*).



To recapitulate, the dry, well-drained, nonsaline land in the upper part of the valley is occupied chiefly by the sagebrush association; the dry saline land near the center is covered with a vegetation of *Kochia* or of shadscale; the land in the lower part of the valley, which is often dry on the surface but has a moist subsoil, bears a mixed vegetation of greasewood and shadscale; while the lowest areas near the lake shore, where the soil is strongly saline to the surface and where during much of the year even the first foot is wet, bear the salt-flat type of vegetation. The grass flats occupy a moist, moderately saline area lying between the two preceding. These relationships are shown in Table XVIII, p. 413, and are graphically represented in figure 13 on p. 412.

In the following pages descriptions are given of the several associations and other plant communities, arranged in the order shown in Table III.

#### SAGEBRUSH ASSOCIATION

##### TOPOGRAPHICAL RELATIONS

The sagebrush association is one of the most important types of vegetation of the Great Basin region. In Tooele Valley (see map, Pl. XLII) it occurs chiefly on the bench lands which skirt the mountains. The best growth of sagebrush (apart from that on the sand hills as described later) is found on the alluvial fans which are situated near the mouths of canyons. In such places the moisture received directly as precipitation is probably supplemented by water from the hills. This type of vegetation extends across the southern end of the valley and probably at one time formed a continuous belt, although fire and cultivation have greatly diminished the area originally occupied, especially on the east side. Farther down the valley, below the main area occupied by this association, sagebrush is found only on sand hills, along drainage channels, and in depressions—places where the moisture conditions are more favorable and more of the alkali has been leached out than in the surrounding areas.

##### BOTANICAL COMPOSITION

This association in its typical form is dominated by *Artemisia tridentata* (Pl. XLIV) as almost the sole woody plant. In less typical phases two or three species of rabbit brush (*Chrysothamnus*) occur.<sup>1</sup> Many species of perennial herbs associate with the sagebrush, especially in those portions of the area which lie nearest the foothills. The following list includes all shrubs and perennial herbs which were noted as belonging to the sagebrush association.

<sup>1</sup> These are never abundant and never attain their maximum size where they occur in the typical sagebrush association in Tooele Valley. They appear more at home where associated with *Artemisia* on the sand hills, and at roadsides and along ditches in areas which were formerly covered with the sagebrush association.

PERENNIAL SPECIES OF THE SAGEBRUSH ASSOCIATION<sup>1</sup>

## Common or frequent

<i>Agropyron spicatum</i> (Pursh) Rydb.	<i>Castilleja linariaefolia</i> Benth.
<i>Eriocoma cuspidata</i> Nutt.	<i>Artemisia tridentata</i> Nutt.
<i>Poa sandbergii</i> Vasey	<i>Chrysothamnus marianus</i> Rydb.
<i>Sitanion jubatum</i> J. G. Smith	<i>Chrysothamnus nauseosus albicaulis</i> (Nutt.) Rydb.
<i>Zygadenus paniculatus</i> Wats.	<i>Chrysothamnus pumilus</i> Nutt.
<i>Eriogonum ovalifolium</i> Nutt.	<i>Erigeron pumilus</i> Nutt.
<i>Opuntia</i> sp.	<i>Gutierrezia sarothrae</i> (Pursh) B. and R.
<i>Malvastrum coccineum</i> (Pursh) Gray	<i>Senecio uintahensis</i> A. Nels.
<i>Phlox longifolia</i> Nutt.	

## Less frequent or rare

<i>Stipa comata</i> Trin. and Rupr.	<i>Thalesia fasciculata</i> (Nutt.) Brit.
<i>Atriplex canescens</i> (Pursh) James	<i>Antennaria dimorpha</i> (Nutt.) T. and G.
<i>Delphinium burkei</i> Greene	<i>Balsamorhiza hirsuta</i> Nutt.
<i>Cowania stansburiana</i> Torr.	<i>Balsamorhiza sagittata</i> (Pursh) Nutt.
<i>Astragalus arietinus</i> Jones	<i>Chaenactis douglasii</i> H. and A.
<i>Astragalus beckwithii</i> T. and G.	<i>Chrysopsis villosa</i> (Pursh) Nutt.
<i>Astragalus utahensis</i> T. and G.	<i>Crepis occidentalis</i> Nutt.
<i>Anogra pallida</i> (Lindl.) Brit.	<i>Layia glandulosa</i> H. and A.
<i>Gaura parviflora</i> Dougl.	<i>Leucelene ericoides</i> (Torr.) Greene
<i>Pachylophus marginatus</i> (Nutt.) Rydb.	<i>Ptilocalais nutans</i> (Geyer) Greene
<i>Lappula caerulea</i> Rydb.	<i>Tetradymia inermis</i> Nutt.
<i>Lappula occidentalis</i> (Wats.) Greene	

Numerous annual and biennial plants occur in this association. By far the most abundant of these are two introduced species, *Bromus tectorum* and alfalfa (*Erodium cicutarium*), which in many places cover the ground among the "sage" bushes with a dense mat of vegetation. The more abundant or otherwise conspicuous annual and biennial plants of the sagebrush association are given in the following list:

## ANNUAL AND BIENNIAL SPECIES OF THE SAGEBRUSH ASSOCIATION

<i>Bromus tectorum</i> L.	<i>Mentzelia laevicaulis</i> (Dougl.) T. and G.
<i>Festuca octoflora hirtella</i> Piper	<i>Anogra albicaulis</i> (Pursh) Brit.
<i>Arabis longirostris</i> Wats.	<i>Phacelia linearis</i> (Pursh) Holz.
<i>Draba</i> sp.	<i>Cryptantha</i> sp.
<i>Sophia filipes</i> (Gray) Heller	<i>Lappula cupulata</i> (Gray) Rydb.
<i>Sophia pinnata</i> (Walt.) Brit.	<i>Lappula subdecumbens</i> (Parry) Nels.
<i>Erodium cicutarium</i> L'Her.	<i>Amsinckia tessellata</i> Gray
<i>Mentzelia dispersa</i> (Wats.) A. Nels.	

## APPEARANCE

The characteristic appearance of the sagebrush association is illustrated in Plate XLIV, figure 1. During the early summer, when their maximum growth is taking place, the sagebrush plants present a silvery

<sup>1</sup> In this and all following lists of species the families are arranged in the sequence of Engler and Prantl (Die Natürlichen Pflanzenfamilien), while the genera are arranged alphabetically under each family.

appearance, due to the hairy covering of the young leaves. From the middle of summer to the following spring the plants having lost many of their leaves and the dark stems being more in evidence, the appearance of the vegetation is decidedly different. Still another aspect is that of the early fall when the *Artemisia* plants are in flower and give a yellowish color to the vegetation. The contrast between the comparatively vivid and varied appearance of the vegetation in early summer and its monotonous aspect during the rest of the year is heightened by the fact that nearly all of the flowering herbs belonging to this association die, at least to the ground, long before the close of the summer.

In some parts of the valley, especially where the soil is sandy, the plants of sagebrush are tall, vigorous, and stand close together. In other and more extensive areas, where the moisture conditions are less favorable, they are scattered and stunted, and the proportion of new growth to old wood is small.<sup>1</sup> The plants, in fact, look as if they were slowly dying in such areas. By far the best growth of *Artemisia tridentata*, is found on the sand hills and along irrigating

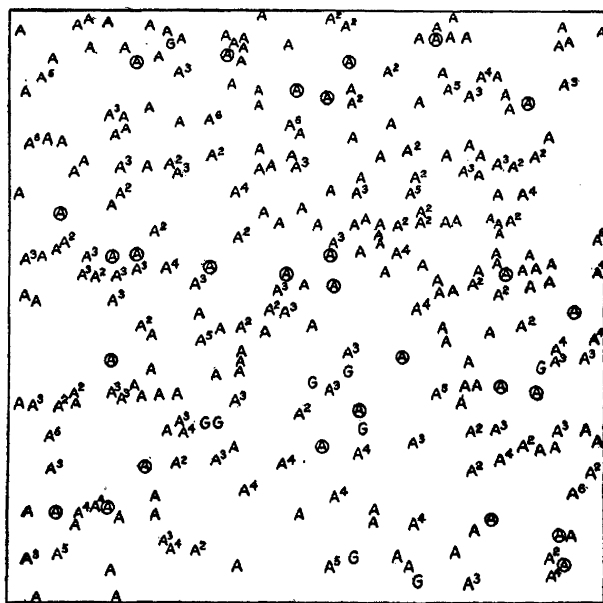


FIG. 3.—A representative 10-meter quadrat of the sagebrush association, showing the location of each individual of *Artemisia tridentata* (A) and of *Gutierrezia sarothrae* (G), these being the only woody species present. The figures show the number of main branches of the *Artemisia* plants and hence indicate their relative size. The absence of a figure indicates that there was only one large stem. A circle around the A indicates a dead plant of *Artemisia*. *Bromus tectorum* was very abundant around the *Artemisia* bushes, and *Sitanion* was also present. These two grasses are not shown on the quadrat.

ditches. In the greater part of the area occupied by this association the plants are from 2½ to 4 feet high. Their frequency is indicated in figure 3, which represents a quadrat<sup>2</sup> platted early in the month of August in a typical portion of this association as it occurs in Tooele Valley.

The associated herbs, although of many species, are not sufficiently numerous individually nor sufficiently large in size to materially affect the aspect of the vegetation, even when they are at the height of their

<sup>1</sup> These slow-growing plants reach a considerable age without attaining a large stem diameter. Twenty-three annual rings were counted in a stem barely 2¼ inches in cross section.

<sup>2</sup> For descriptions of the method of quadrats, see Clements, F. E., *Research Methods in Ecology*, Lincoln, 1905, p. 161-176; and also his *Plant Physiology and Ecology*, New York, 1907, p. 202-210.

growth and blossoming. Moreover, they are apt to be partly hidden, owing to their habit of growing close among the stems of the sagebrush. After midsummer most of the herbaceous species die, at least to the ground, and during the rest of the year typical areas when viewed from a little distance appear to contain no species other than *Artemisia tridentata*.

#### PHYSICAL CONDITIONS INDICATED

The soils occupied by the sagebrush association, which consist largely of products of erosion deposited upon the bed of the ancient Lake Bonneville, are rather coarse in texture and often contain much gravel. All available data concerning the moisture conditions and salt content of the soil in typical portions of this association as it occurs in Tooele Valley are given in Table IV.

TABLE IV.—Sagebrush association: Moisture conditions and salt content of the soil in typical areas.<sup>1</sup>

Item.	Depth of soil (feet.)	Date of collection.												Average.
		June.									August.			
		3	5	5	15	15	15	15	17	17	3	7	7	
No. of sample		15	25	27	36	37	38	39	40	41	104	111	115	
Moisture equivalent	1			13.1	15.9	16.7	12.6	15.7	18.4			12.1	9.3	14.2
	2			15.6	17.4	17.7	22.9	14.7	19.1			8.9	9.0	15.6
	3			23.8	13.3	22.2	24.5		15.2			7.5	9.0	16.5
	4			19.6	11.6		23.4						8.7	15.8
Wilting coefficient	1			7.1	8.6	9.1	6.8	8.5	10.0			6.6	5.0	7.7
	2			8.5	9.4	9.6	12.4	8.0	10.4			4.8	4.9	8.5
	3			12.9	7.2	12.0	13.3		8.2			4.1	4.9	8.9
	4			10.6	6.3		12.7						4.7	8.6
Moisture content above or below the wilting coefficient	1			- .7	-1.4	-3.0	-1.2	-3.7	-5.0					-2.5
	2			+3.1	+ .4	-1.5	+ .3	-2.5	-4.1					- .6
	3			-4.4	+ .7	-4.4	+3.7		-3.2					-1.3
	4			-1.0	0		+4.0							+1.0
Salt content	1	0.03	0.04	.03	.04	.03	.03	.03	0.03	0.03	0.03	.03	.03	.03
	2	.03		.03	.04	.06	.03	.03	.03	.03	.03	.02	.03	.03
	3			.08	.03	.12	.05		.02			.02	.03	.05
	4			.10	.03		.12						.05	.07
	5			.05			.10							.07

<sup>1</sup> All data in this table are stated in percentages of the dry weight of the soil. The moisture contents with a plus sign (+) represent moisture available for growth (above the wilting coefficient), while those with a minus sign (-) represent a corresponding deficit of available moisture (below the wilting coefficient).

**SOIL, MOISTURE.**—Typical sagebrush land is characterized by a rather light texture of the soil, as is indicated by the relatively low moisture equivalent. In such soil water penetrates readily to considerable depths, and the run-off must be small. Consequently, although the moisture-holding capacity is low, the total quantity of water available to deep-rooted plants is considerable.

The rapid growth of the sagebrush plants in the early part of the summer results in a speedy exhaustion of the moisture available for growth, and in most years the water content of the soil to the depth reached by the roots is probably reduced by midsummer to below the

wilting coefficient in much the greater part of the area occupied by this association. That this is the case is strongly indicated by the fact that most samples of soil collected during the month of June, 1912, showed very little or no moisture above the wilting coefficient to a depth of 4 feet (Table IV).

In places where the conditions for the reception of water and removal of alkali salts are more than usually favorable—e. g., along drainage channels, in depressions, and in places where the soil has been loosened by burrowing animals—there is probably available moisture within reach of the roots during a longer period. *Artemisia tridentata* was not seen growing under natural conditions where the water table is near the surface of the soil.

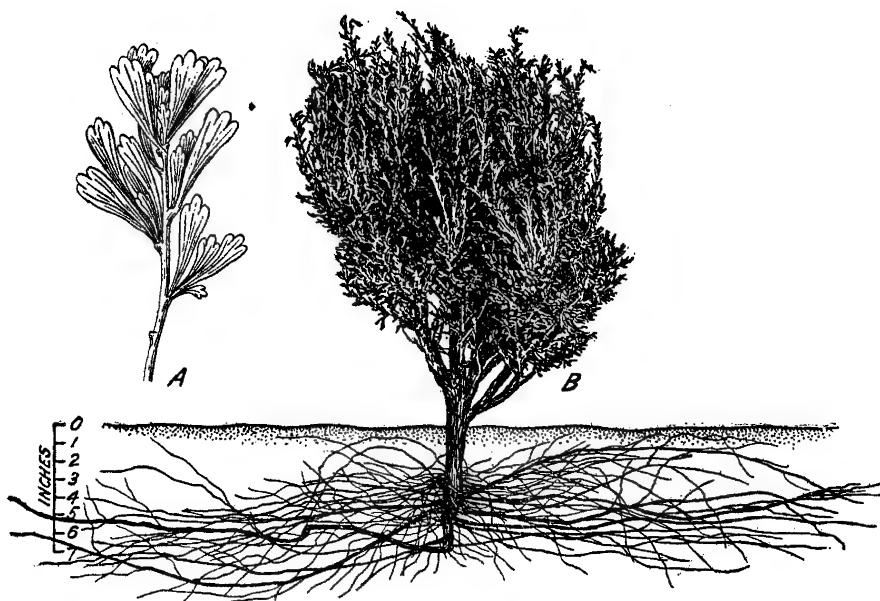


FIG. 4.—*Artemisia tridentata* (sagebrush): A, Detail showing the wedge-shaped, 3-toothed leaves by which this plant is easily recognized; B, a small plant growing where hardpan occurred, showing the deflection of the taproot from a vertical to a horizontal direction after reaching a depth of 5 inches.

Optimum soil-moisture conditions for the growth of *Artemisia tridentata* are rarely realized in Tooele Valley. This is shown by the much larger size and more vigorous appearance of the plants which grow on sand hills and along irrigating ditches. In many places the maximum depth reached by the roots is only from 18 to 30 inches and is marked by the presence of a hardpan consisting of coarse gravel cemented by calcareous material. The depth at which this hardpan is formed probably represents the limit of penetration of the rain water, and consequently most of the roots of the sagebrush do not penetrate farther. The shallowness of the moisture-holding layer of the soil greatly reduces the absolute quantity of moisture available for growth. The effect is shown in the thin stand and in the small size and sickly appearance of the plants (fig. 4). The eastern part of the valley, where most of the dry-

farming area is situated, was in all probability once covered with sagebrush vegetation, although few traces of it now remain. Here the conditions were probably more favorable for the growth of this plant than in most of the area still occupied by it.

**SALINITY.**—Reference to Table IV shows that in the typical sagebrush land of Tooele Valley the salt content of the soil is extremely low—lower, in fact, than in many soils of humid regions. Near the lines of contact with other associations, however, *Artemisia tridentata* frequently grows where much salt is present at a depth of 30 or 40 inches. In such places the saline subsoil is an effectual barrier to the penetration of the roots, the depth of soil from which the plant must extract its entire supply of water is correspondingly limited, and as a result the plants are scattered, very small, and give every appearance of suffering from drought.

An excellent example of this condition was observed on the west side of the valley, where in a spot of considerable size the plants of *Artemisia* were widely spaced, rarely more than 2 feet high, and had many dead branches. Samples of soil collected in this spot on June 3 gave salt contents and moisture contents as follows:

TABLE V.—Salt content and moisture content (above or below the wilting coefficient) at different depths of the soil where the sagebrush was small and suffering.

Depth.	Salt content.	Moisture content above or below wilting coefficient.
<i>Feet.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1	0.05	—2.0
2	.18	—2.2
3	.53	—3.4
4	.64	+ .8
5	.59	

The roots of the plant alongside this boring penetrated to a depth of only about 2 feet, at which point the taproot had died, and development was continued by horizontal laterals. The feeding roots were mostly confined to the first foot of the soil.

The most extreme condition as regards salinity which was noted at any point in Tooele Valley where *Artemisia tridentata* grew was in a small pocketlike depression among the sand hills where salts had accumulated as a result of seepage from the surrounding dunes and where very small sickly plants of sagebrush grew in company with greasewood (*Sarcobatus*) and *Kochia*. The salt contents were as follows: First foot, 0.16 per cent; second foot, 0.51 per cent; third foot, 0.67 per cent; fourth foot, 0.66 per cent. The presence here of living plants of sagebrush is doubtless explained by the fact that large seed-producing plants of this species were growing on the surrounding dunes and that the salt content of the

surface soil was not high enough to prevent the germination and seedling growth of the *Artemisia*.

SUMMARY OF PHYSICAL CONDITIONS.—The observations made in Tooele Valley lead to the conclusion that in this area a good stand and growth of sagebrush indicates (1) a rather coarse textured, readily permeable soil, with low run-off and good underdrainage (water table low); (2) a depth of soil of at least 3 feet, in which water can be stored and into which the roots of plants may easily penetrate; (3) at least 3 feet of soil free from alkali salts in quantity sufficient to injure ordinary crop plants.

#### ADAPTATIONS TO THE PHYSICAL CONDITIONS

The herbaceous species of the sagebrush association are for the most part shallow rooted, and, hence, are dependent upon the moisture of the upper soil. The great majority of them grow so rapidly during the spring and early summer that they are able to complete their development and ripen seed before the water content of the first foot or two of the soil has been exhausted to the wilting coefficient. When this occurs, they die, at least to the ground.<sup>1</sup> After the middle of July few living plants except sagebrush are visible in typical areas of this association.

The dominant species, *Artemisia tridentata* (sagebrush) is able to continue growth during a longer period. As shown in figure 5 and in Plate XLIV, figure 2, it possesses a "generalized" type of roots<sup>2</sup>—i. e., a highly developed system of laterals in the upper soil and also a deeply penetrating taproot. The former are admirably adapted for securing the moisture which penetrates only to a small depth during light rains and for which in spring and early summer this plant must compete with the numerous associated annual and perennial herbs. By means of its taproot the plant can also avail itself of moisture stored at greater depths<sup>3</sup> in the readily permeable soils which are preferred by this association.

The great development of superficial lateral roots favors rapid growth so long as abundant moisture is present in the upper soil, while the deep penetration of the taproot permits the plant to continue growth at a slower rate long after most of the herbaceous species of this association have withered away. In typical areas of sagebrush vegetation as represented in Tooele Valley (Pl. XLIV, fig. 1) the available moisture is probably exhausted before the end of the summer in all depths of soil

<sup>1</sup> They are for the most part "drought escaping" rather than "drought enduring." See Kearney, T. H., and Shantz, H. L., The water economy of dry-land crops, U. S. Dept. Agr., Yearbook, 1911, p. 354-357, 1912.

<sup>2</sup> See Cannon, W. A., The Root Habits of Desert Plants, Washington, p. 87, 1911. (Carnegie Inst., Washington, Pub. 131.)

<sup>3</sup> Plate XLIV, figure 2, reproduced from a photograph taken in the vicinity of Nephi, Utah, shows the taproot of *Artemisia tridentata* extending vertically to a depth of over 15 feet. The root penetration of this plant under optimum conditions was not studied in Tooele Valley, but it is unlikely that in most of the area there occupied by this association the roots reach so great a depth. In this locality the deepest rooting plants are doubtless those which grow on the sand hills.

reached by the *Artemisia* roots. The plants then lose many of their leaves and make no further growth until the following spring.<sup>1</sup>

The total transpiring surface is small in proportion to the size of the plant, especially where the physical conditions are least favorable, and this helps to prevent rapid exhaustion of the available soil moisture. The limited amount of new growth made during exceptionally dry seasons

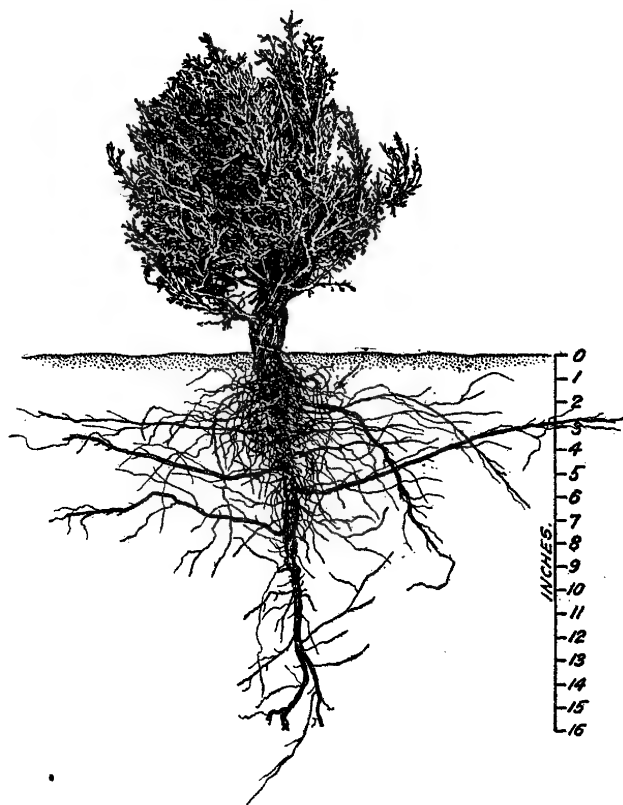


FIG. 5.—A small plant of sagebrush (*Artemisia tridentata*), showing the deeply penetrating taproot and good development of superficial lateral roots typical of this species.

diminishes the danger of death from drought. Another circumstance which serves as a protection from this danger is the thinness of the stand. Even on the sand hills, where the conditions are most favorable for their growth, the sagebrush plants are rarely crowded. In proportion as the soil-moisture conditions depart more and more from the optimum for this species, the plants are farther and farther apart. Each individual (Pl. XLIV, fig. 1) is surrounded by a space of ground which is bare during the greater part of the year, although producing a few shallow-rooted herbaceous plants in spring and early summer. The wide spacing of the plants is indicated in figure 3.

#### EFFECTS OF DISTURBING FACTORS: SUCCESSIONS

During the summer and autumn large areas of sagebrush are often burned over. The fire consumes the dry herbaceous growth and the sagebrush plants are usually burned to the ground. They do not sprout up from the old stumps, and the result is usually the complete removal of the *Artemisia*. In the following year a mat of herbaceous vegetation, composed chiefly of *Bromus tectorum* and *Erodium cicutarium*, covers the

<sup>1</sup> Sagebrush is therefore to be classed as a "drought-enduring" species. See Kearney and Shantz, op. cit., p. 354, 355.



ground among the blackened stumps. After a few years *Gutierrezia* is likely to become the dominant plant on these burned-over areas. (Pl. XLV, fig. 1.) This, in turn, is followed by the sagebrush, which gradually reestablishes itself.

In sagebrush land which has been plowed up and subsequently abandoned, the removal of the shrubs favors the development of various annual and biennial weeds, such as *Bromus tectorum*, alfilaria (*Erodium cicutarium*), pigweeds (species of *Amaranthus*), Sunflower (*Helianthus annuus*), wild tomato (*Solanum triflorum*), vervain (*Verbena bracteosa*), etc. As time goes on, *Gutierrezia sarothrae*, a small, much-branched, yellow-flowered composite, often becomes established and maintains itself for a period which is short or long accordingly as the conditions are more or less favorable for the reestablishment of the sagebrush. Sooner or later the *Artemisia* reappears (Pl. XLV, fig. 2), and unless fire or some other disturbing factor intervenes, the territory is eventually reconquered by the original association. (Pl. XLV, fig. 3.)

The succession after either fire or breaking may be shortened, *Artemisia* following immediately after the annual weed stage, without the intervention of *Gutierrezia*. As a rule, however, the succession comprises (1) a growth of annual and biennial weeds, (2) a growth of the perennial *Gutierrezia*, and (3) the return of the original sagebrush vegetation.

Grazing does not materially alter the sagebrush vegetation, although diminishing the numbers of many of the herbaceous species. *Artemisia tridentata* itself is rarely eaten and is, in fact, benefited by grazing, since the plants which compete with it for the soil moisture are thereby removed.

#### VARIATIONS FROM THE TYPICAL ASSOCIATION

**SAGEBRUSH WITH KOCHIA AND WITH SHADSCALE.**—Near the lower limit of the main area occupied by sagebrush this association comes into contact with the *Kochia* and shadscale associations, and the dominant species of the three associations often grow together in a mixed community. The plants of *Artemisia* which push out farthest into areas occupied by these other associations are confined to drainage channels, depressions, and the vicinity of animal burrows. In such places the conditions as to soil moisture are more favorable and the greater penetration of the rain water has leached the salts into lower depths of soil than is generally the case in *Kochia* and shadscale land. But along the frontiers of these associations scattered, small, and sickly looking plants of *Artemisia* mingle directly with *Kochia* or with shadscale.

Borings made where *Artemisia tridentata* and *Atriplex confertifolia* grow side by side invariably showed the presence of salts in the second, or, at the deepest, in the third foot of the soil. (Table VI.) The sagebrush roots are unable to penetrate this saline subsoil, and the total quantity of water available for the growth of this plant is correspondingly limited.

TABLE VI.—Salt content of the soil at points where *Artemisia tridentata* and *Atriplex confertifolia* grew side by side.

Depth of soil.	Salt content in boring No.—			
	59	60	95	96
Feet.	Per cent.	Per cent.	Per cent.	Per cent.
1	0.06	0.08	0.12	0.04
2	.27	.53	.53	.04
3	.....	.....	1.02	.76
4	.....	.....	1.36	.....

SAGEBRUSH WITH JUNIPER.—The Utah juniper (*Juniperus utahensis*) is abundant on the lower slopes of the mountains and also pushes down into the upper part of Tooele Valley, where it occurs scatteringly in the midst of areas occupied by the typical sagebrush association (see background of Pl. XLIV, fig. 1), as well as on the sand hills. The presence of juniper away from the sand hills usually indicates a stonier soil than that on which the typical sagebrush association occurs.

#### SAND-HILL MIXED ASSOCIATION

##### TOPOGRAPHICAL RELATIONS

The sand-hill mixed association covers a limited area towards the south end of the valley, lying directly in the path of the winds from the southwest which sweep over the low divide separating Tooele Valley from Rush Valley. Even when the air is nearly motionless in other parts of the valley, a sandstorm may often be seen blowing in this quarter. The sand is mostly heaped in dunes, which form more or less continuous ridges having a general north and south trend. In places where "blow-outs" have taken place the ground is sometimes bare, but for the most part it is fairly well covered with vegetation.

##### APPEARANCE AND BOTANICAL COMPOSITION

As is usually the case in arid regions, the vegetation of the sand hills is characterized by the presence of a large number of species—far more than occur in any other plant association of Tooele Valley. The appearance of the vegetation as viewed a short distance away is determined by the presence of a few woody species, notably sagebrush (*Artemisia tridentata*) and juniper (*Juniperus utahensis*). Sagebrush is much the most abundant of the woody plants of the sand hills, and the individual plants of this species which grow there are the largest and thriftiest found anywhere in Tooele Valley under natural conditions.

The Utah juniper is fairly abundant on the sand hills. It occurs as a large shrub or small tree, rarely exceeding 10 feet in height. Two species of rabbit brush (*Chrysothamnus nauseosus albicaulis* and *C. pumilus*) are also common, while the remaining woody species of this

association, *Atriplex canescens*, *Grayia spinosa*, *Sarcobatus vermiculatus*, and *Purshia tridentata*, are relatively infrequent. The predominance of woody plants distinguishes the sand-hill association of Tooele Valley from the corresponding type of vegetation in the Great Plains east of the Rocky Mountains.<sup>1</sup>

Next to the shrubs, perennial herbs are the most important members of this association. Noteworthy among these are two characteristic sand-loving species, *Psoralea lanceolata* and *Abronia salsa*. Certain bunch grasses, *Eriocoma cuspidata*, *Stipa comata*, and *Agropyron spicatum*, are also important constituents of this vegetation. A few annual and biennial species are to be seen during the first weeks of summer, but the plants are too small and too short lived to greatly influence the appearance of the vegetation.

The following list includes the more important species noted as occurring in the sand-hill mixed association:

## PERENNIAL SPECIES

*Juniperus utahensis* (Englm.) Lemm.  
*Agropyron spicatum* (Pursh) Rydb.  
*Eriocoma cuspidata* Nutt.  
*Stipa comata* Trin. and Rupr.  
*Eriogonum ovalifolium* Nutt.  
*Eriogonum kearneyi* Tidestrom  
*Atriplex canescens* (Pursh) James  
*Eurotia lanata* (Pursh) Moq.  
*Grayia spinosa* (Hook.) Moq.  
*Sarcobatus vermiculatus* (Hook.) Torr.  
*Abronia salsa* Rydb.

*Purshia tridentata* (Pursh) DC.  
*Psoralea lanceolata* Pursh  
*Gilia pungens* (Torr.) Benth.  
*Lappula occidentalis* (Wats.) Greene  
*Castilleja linariaefolia* Benth.  
*Artemisia tridentata* Nutt.  
*Chrysothamnus nauseosus albicaulis* (Nutt.)  
 Rydb.  
*Chrysothamnus pumilus* Nutt.  
*Layia glandulosa* H. and H.  
*Senecio uintahensis* A. Nels.

## ANNUAL AND BIENNIAL SPECIES

*Abronia cycloptera* Gray  
*Eriogonum cernuum* Nutt.  
*Lepidium pubescens* A. Nels.  
*Erodium cicutarium* L'Her.

*Gilia leptomeria* Gray  
*Crytanthe* sp.  
*Lappula* sp.

## PHYSICAL CONDITIONS INDICATED

The soil is nearly pure sand and is easily moved by the wind. The conditions for penetration of the total rainfall are excellent and the run-off is negligible. The great depth of loose soil is favorable to storage of water during a long period after rains. Only one soil boring in this association was made (June 3), but the location was apparently in all respects a typical one and the resulting data (Table VII) probably represent the average conditions of moisture and salt content of the soil where this type of vegetation occurs.

<sup>1</sup> Shantz, H. L., Natural vegetation as an indicator of the capabilities of land for crop production in the Great Plains area. U. S. Dept. Agr., Bur. Plant Indus. Bul. 201, p. 58-60. 1911.

TABLE VII.—*Sand-hill mixed association: Moisture conditions and salt content of the soil in a typical area.*<sup>1</sup>

Depth (feet).	Moisture equivalent.	Wilting coefficient.	Moisture content above or below the wilting coefficient.	Salt content.
1	9.2	5.0	-0.1	0.03
2	9.7	5.3	+0.7	.03
3	6.2	3.4	+1.1	.03
4	5.8	3.1	+1.3	.04
5	.....	.....	.....	.01
6	.....	.....	.....	.01

<sup>1</sup> All data are in percentages of the dry weight of the soil.

If the data given in Table VII may be taken as representative, land occupied by this association is characterized by the following soil conditions: (1) A low moisture-holding capacity, as indicated by the low moisture equivalents, (2) available moisture present, at least during the fore part of the summer, at a depth attainable by the more deeply penetrating roots, and (3) a very low salt content.

#### ADAPTATIONS TO THE PHYSICAL CONDITIONS

The soil-moisture conditions of the sand hills are obviously such as to favor plants with deeply penetrating roots, and, accordingly, large woody plants are predominant in this association. Sagebrush, the most abundant woody species, is noteworthy for the great depth reached by its taproot when the conditions are favorable. Of the herbaceous species of this association, some have a well-developed taproot, while others produce an abundance of superficial roots. The shallow-rooted herbs, being dependent upon the moisture of the surface soil, mostly complete their growth and ripen seed early in the summer. Certain of the perennial herbs, notably *Psoralea lanceolata*, spread by slender, creeping rootstocks and can therefore withstand frequent burial. This plant is excellently adapted to colonizing the blow-outs and may be regarded as the pioneer plant of the moving sands.

#### KOCHIA ASSOCIATION<sup>1</sup>

##### TOPOGRAPHICAL RELATIONS

The *Kochia* association (Pl. XLVI) occupies a narrow and nearly continuous belt which extends across the valley along the lower boundary of the sagebrush area and lies between the latter and the shadscale area. (See map, Pl. XLII.) This type of vegetation likewise occurs as islands of greater or less extent scattered through the sagebrush zone well

<sup>1</sup> While this plant association is one of the most important in Tooele Valley, it appears to be a much less prominent feature of the vegetation in other portions of central and western Utah.

toward the head of the valley. (Pl. XLIII, fig. 2.) The boundaries between the areas occupied by the *Kochia* and by the sagebrush associations are usually very sharply defined. Equally abrupt is the change in salt content of the soil, as is well exemplified by the results of borings which were made on either side of the line of contact shown in Plate XLVI, figure 1. The location of the boring in the sagebrush association is marked by the position of the soil tube shown in the illustration. The two borings were only 20 feet apart. The results are given in Table VIII.

TABLE VIII.—Salt content (in percentages of the dry weight of the soil) on either side of a line of contact between the sagebrush and *Kochia* associations.

Depth (feet).	Sagebrush.	<i>Kochia</i> .
1	0.03	0.31
2	.03	1.49

On the other hand, the boundaries between the *Kochia* and shadscale associations are usually indefinite.

*Kochia vestita*, sometimes locally known as "white sage," which is the dominant species of the *Kochia* association, is also a frequent component of the shadscale and greasewood-shadscale associations, reaching the shore of Great Salt Lake with the latter association. But the small size of the plants as compared with those of shadscale and of greasewood makes *Kochia* an inconspicuous member of these associations.

In typical portions of this association *Kochia vestita* is almost the only species of flowering plant, except that where grazing animals are kept off the land, a small grass, *Poa sandbergii*, is very abundant. Few other species occur, and these are seldom represented by numerous individuals. The following list includes all species of flowering plants which were noted as occurring in typical areas of the *Kochia* association:

#### BOTANICAL COMPOSITION

*Kochia vestita* (Wats.) A. Nels.  
*Poa sandbergii* Vasey  
*Erodium cicutarium* L'Her.  
*Lepidium* sp.

*Sphaerostigma pubens* (Wats.) Rydb.  
*Opuntia* sp.  
*Gutierrezia sarothrae* (Pursh) B. and R.

#### APPEARANCE

The *Kochia* association is the most uniform in appearance of the types of vegetation occurring in this valley (Pl. XLVI, fig. 2). It is virtually a 1-species association. The height of the plants varies but slightly over large areas and usually does not exceed 6 inches. Owing to the low growth and the hairiness of the plants (see text fig. 7 and Pl. XLVI, fig. 3), an area of *Kochia* presents at a little distance the homogeneous appearance of a gray blanket. Even at a distance of several miles, the strips

and islands occupied by this plant are easily distinguishable from surrounding areas of sagebrush vegetation (Pl. XLIII, fig. 2). The contrast is especially striking in spring and early summer when the sagebrush has a silvery green color, which is quite distinct from the dull gray of the *Kochia*.

When viewed closely (Pl. XLVI), the plants are found to be separated by patches of bare ground which vary in size as the physical conditions are more or less favorable. In a more distant view the light ashy color of the soil occupied by this association blends with the color of the plants themselves, and this tends to create the impression

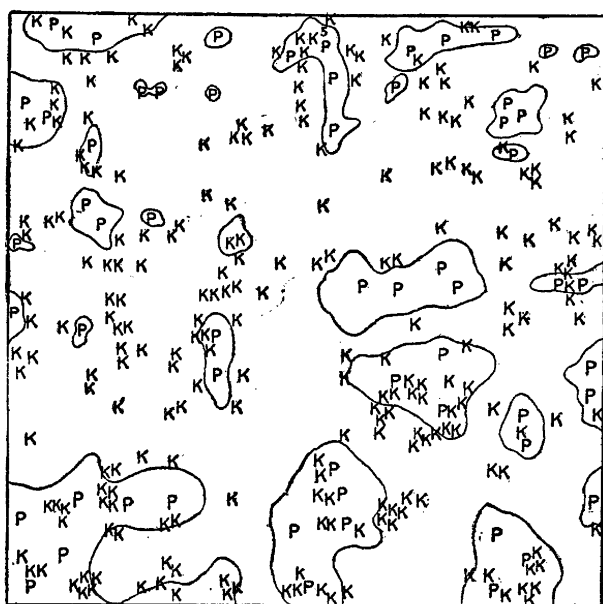


FIG. 6.—A representative 20-meter quadrat of the *Kochia* association, showing the location of each tuft of *Kochia* (K) and of each matlike colony of *Poa sandbergii* (P).

distribution of the plants of *Kochia* and of *Poa* in a typical, unmodified quadrat of this association is shown in figure 6.

#### PHYSICAL CONDITIONS INDICATED

The type of land occupied by the *Kochia* association in its typical form is uniform and well defined. The soil is remarkably homogeneous to a depth of several feet, fine in texture, and close in structure. Unlike sagebrush and shadscale lands, there is usually little gravel present. The smooth, polished condition of the surface after it has been wet indicates that this soil puddles readily. The conditions for the penetration of water are, therefore, unfavorable, and the run-off is doubtless high.

that the plant covering is dense. In fenced areas occupied by this association the color is modified to a yellowish or brownish gray during a few weeks in the early part of the summer, owing to the abundant fruiting heads of a small grass, *Poa sandbergii* (Pl. XLVI, fig. 3). But most of this land is grazed by sheep, which soon extirpate the grass or at least prevent its flowering, while leaving the *Kochia* practically undisturbed. The

TABLE IX.—*Kochia* association: Moisture conditions and salt content of the soil in typical areas.<sup>1</sup>

Item.	Depth of soil (feet).	Date of collection.										Average.	
		June.					July.			August.			
		1	1	7	18	18	3	5	12	3	7		
No. of sample.....		4	5	32	44	45	65	70	78	105	116		
Moisture equivalent.....	{	1	23.0	35.8	21.7	26.0	24.7	23.9	25.8				
		2	25.5	25.2	30.3	26.4	29.4	25.3	27.0				
		3	33.3	35.0	29.4	34.5	36.8	32.4	33.5				
		4	31.4	34.2	24.4	35.0	34.3	32.5	31.9				
Wilting coefficient.....	{	1	12.5	19.5	11.8	14.1	13.4	13.0	14.0				
		2	13.9	13.7	16.5	14.3	16.0	13.7	14.7				
		3	18.1	19.0	16.0	18.7	20.0	17.6	18.2				
		4	17.0	18.6	13.2	19.0	18.6	17.7	17.3				
Moisture content above or below wilting coefficient.....	{	1	-1.8	-9.2	-4.2	-6.2	-5.8	.....	-5.4				
		2	-1.5	-2.6	-.3	+1.6	-6.1	.....	-1.8				
		3	-5.4	-5.3	-2.0	-2.0	+2.0	.....	-2.5				
		4	-3.2	-5.0	-1.9	+1.6	-3.3	.....	-2.4				
Salt content.....	{	1	.05	.08	.04	.22	.14	.07	.06	.18	.31	.05	.12
		2	.30	.16	.14	1.20	.30	.47	.32	.80	1.49	.31	.55
		3	.82	.60	.52	1.14	1.56	1.10	.88	1.36	1.36	.92	1.02
		4	.....	.70	.78	1.02	.....	1.43	.97	.....	1.76	1.10	1.11

<sup>1</sup> All data in this table are stated in percentages of the dry weight of the soil. The moisture contents with a plus sign (+) represent moisture available for growth (above the wilting coefficient), while those with a minus sign (-) represent a corresponding deficit of available moisture (below the wilting coefficient).

**SOIL MOISTURE.**—The moisture equivalents given in Table IX indicate that the moisture-holding capacity of the soil is much higher in *Kochia* land than in sagebrush land. The moisture contents in typical areas show that as early as the first of June, 1912, the soil to a depth of 4 feet was usually devoid of water available for plant growth. The deficit was usually greatest in the surface foot, partly no doubt because of surface evaporation and partly because of the shallow-rooting habit of *Kochia vestita*.

**SALINITY.**—The soil in typical *Kochia* land, at least in Tooele Valley, is usually free from an injurious quantity of salts in the surface foot. On the other hand, the second foot is usually, and the third and fourth feet are almost invariably, highly saline. In places where the surface foot contains much salts the plants of *Kochia* are scattered and stunted.

There is some evidence that the presence of *Kochia* vegetation, although in the great majority of cases associated with a strongly saline subsoil, does not invariably indicate such a condition. In the upper part of Tooele Valley an island of *Kochia* (Pl. XLIII, fig. 2) several acres in extent, in the midst of the sagebrush zone, was found to be underlain at a depth of 30 inches by a gravelly hardpan. The soil just above this stratum contained only about 0.2 per cent of readily soluble salts. It would seem that here the presence of hardpan rather than of salts had caused the *Artemisia* to give place to *Kochia*.

The conclusion seems warranted that the presence of the *Kochia* association to the exclusion of sagebrush is determined by the occurrence of 1 or at most 2 feet of soil free from an excess of salts, underlain by a subsoil which is strongly saline or which for some other reason is unfavorable to deep penetration of roots.

**SUMMARY OF PHYSICAL CONDITIONS.**—In Tooele Valley the land occupied by the *Kochia* association is distinguished from that occupied by the sagebrush association by its finer texture, its tendency to puddle at the surface and, hence, resist the penetration of water, and its higher moisture-holding capacity, and also by the limitation of the depth in which the roots can freely develop to not more than 24 inches, the obstacle to deeper penetration being usually the high salt content of the subsoil.

#### ADAPTATIONS TO PHYSICAL CONDITIONS

Since *Kochia vestita* is the only very important species of the *Kochia* association, the structure of this plant alone need be considered in its

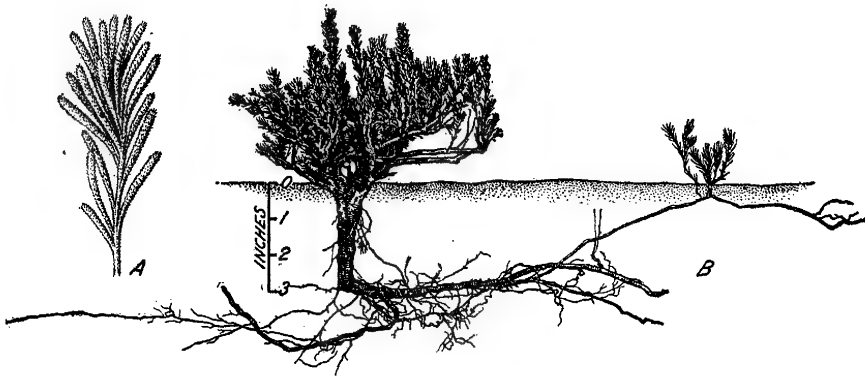


FIG. 7.—*Kochia vestita*: A, Detail, showing the narrow, hairy leaves; B, a plant showing the shallow root system and the propagation by root shoots.

relations to the physical conditions. The underground portion of the plant (fig. 7) is well adapted to the comparatively small depth of soil from which the total supply of water must be obtained. *Kochia vestita* spreads by means of long, slender-branching root shoots, which extend almost horizontally for distances of 10 feet or more, and often at a depth of only 3 inches from the surface of the soil. At intervals clusters of vertical shoots are sent up, and, hence, the plants above ground appear as isolated, unconnected clumps. In typical portions of this association the feeding roots are limited to the first 12 to 18 inches of the soil, the depth which is usually free from excessive quantities of alkali salts.

At one point where the root distribution was studied with special care, living roots were traced to a depth of about 18 inches, and at that depth the soil contained 0.9 per cent of salts, while at a depth of about 21 inches there was 1.6 per cent. Below this depth traces of dead roots were observed. Excavation at another point, where the first 6 inches



of the soil contained about 0.15 per cent of salts, the second 6 inches 0.36 per cent, and the second foot 1.2 per cent, showed only dead roots below the depth of 12 inches. A possible explanation of these circumstances would be that in some past period of exceptionally heavy rainfall the salt had been washed down to an unusually low depth and that the growth of the roots had kept pace. In subsequent years an upward movement of the salts would have resulted in the death of the deeper roots.

The total quantity of water available for growth in Kochia land is probably less than in any other type of land in the valley. The quantity of organic matter produced is also less, and although the plants often remain alive throughout the greater part of the summer the total quantity of water transpired is necessarily small.

*Poa sandbergii*, the only other abundant species of this association, is a shallow-rooted grass, which ripens its seeds and withers to the ground early in the summer. It is clearly dependent upon the moisture available in the surface soil.

#### EFFECTS OF DISTURBING FACTORS: SUCCESSIONS

Where Kochia land has been plowed so as to completely destroy the original vegetation and subsequently has been abandoned, the reestablishment of the Kochia probably takes place rather slowly. When the "breaking" has been less thorough and a few plants have been left alive, the reestablishment of the Kochia proceeds more rapidly. The intervening stage of annual weeds or of *Gutierrezia*, such as occurs when the vegetation has been removed from sagebrush land, apparently does not follow after breaking on Kochia land.

Grazing is general in Tooele Valley, where many sheep are wintered. Kochia land is especially suitable for pasturage, being relatively level and free from spiny shrubs. The Kochia plants themselves are usually not much injured by grazing, but the associated grass (*Poa sandbergii*) is eaten to the ground and is often almost wholly eradicated.

#### VARIATIONS FROM THE TYPICAL ASSOCIATION

KOCHIA WITH SAGEBRUSH.—As was pointed out above, *Artemisia tridentata* penetrates Kochia areas along drainage channels and in other places where the soil-moisture conditions are more favorable and the salt content smaller than in typical Kochia land. When associated with sagebrush, the plants of Kochia are much larger and more vigorous than where this plant occurs in the pure association.

KOCHIA WITH SHADSCALE.—On the lower edge of the Kochia zone plants of shadscale appear, scatteringly at first, then in greater numbers, until finally the two species are found mingled together in approximately equal proportions over large areas. The shadscale, being much the larger plant, is alone visible at a short distance, even where it is numerically

not superior to the *Kochia*. The line of demarkation between the *Kochia* and shadscale associations is never a sharp one, and this conforms with the fact that the physical conditions indicated by the two types of vegetation are similar.

#### SHADSCALE ASSOCIATION

##### TOPOGRAPHICAL RELATIONS

The shadscale association is one of the most characteristic and important of the Great Basin region. In the Tooele Valley (see map, Pl. XLII) it occupies a wide belt across the middle part of the valley, just below the *Kochia* belt, extending farthest northward along the base of the Stansbury Range. The dominant species as a constituent of the greasewood-shadscale association extends to the edge of the grass flats and beyond that area occupies ridges and hummocks on the salt flats which border Great Salt Lake. Isolated small patches of pure shadscale also occur within the area mapped as salt flat.

##### BOTANICAL COMPOSITION

The most abundant plant of this association is the species of saltbush (*Atriplex confertifolia*) which is commonly known as shadscale, from the shape of the scalelike bracts which envelop the fruits. (See fig. 9.) The number of associated species is much smaller than in the sagebrush association, and those which occur are usually represented by fewer individuals. The plants which were noted as occurring in this association are the following:

##### PERENNIAL SPECIES

<i>Poa sandbergii</i> Vasey	<i>Opuntia</i> sp.
<i>Sitanion minus</i> Smith	<i>Lappula occidentalis</i> (Wats.) Greene
<i>Allium acuminatum</i> Hook.	<i>Artemisia spinescens</i> Eat.
<i>Atriplex confertifolia</i> (Torr.) Wats.	<i>Chrysothamnus marianus</i> Rydb.
<i>Eurotia lanata</i> (Pursh) Moq.	<i>Tetradymia glabrata</i> Gray
<i>Kochia vestita</i> (Wats.) A. Nels.	<i>Tetradymia spinosa</i> H. and A.

##### ANNUAL AND BIENNIAL SPECIES

<i>Bromus marginatus seminudus</i> Shear	<i>Cryptantha multicaulis</i> A. Nels.
<i>Lepidium jonesii</i> Rydb.	<i>Oreocarya shantzii</i> Tidestrom
<i>Thelypodium elegans</i> Jones	<i>Townsendia watsonii</i> Gray

##### APPEARANCE

The general appearance of the shadscale association (Pl. XLVII, fig. 1) is due almost entirely to the dominant species. *Atriplex confertifolia* as found in Tooele Valley is a rounded bush, usually about 18 inches in height and also in diameter, with rigid, spiny branches and harsh dry-looking foliage. (See fig. 9.) The individual plants tend to form low hummocks, the soil immediately about them being held by the partly

recumbent, twisted branches, while the bare ground between is subject to blowing.

The prevailing color is a dull grayish brown, turning to reddish brown in autumn. Plants growing in depressions, where the moisture conditions are exceptionally favorable, have a bluish hue. Viewed from a short distance the association gives the impression of extreme monotony and lifelessness.

The distribution of the plants is indicated in figure 8, which represents a quadrat 10 meters square, in a typical portion of this association. Where the conditions are most favorable, the plants have a fairly vigorous appearance and cover somewhat more than half the ground, the stand being frequently more dense than is usually the case in the sagebrush association. In much the greater part of the area, however, the proportion of bare ground is greater and the plants seem to be having a hard struggle to maintain life, many of their branches being dead or dying. (Pl. XLVII, fig. 1.) No other vegetation in this valley gives the impression of being so nearly conquered by the environment. Even the few species which grow on the salt flats have the appearance of finding their habitat more congenial.

The associated species contribute scarcely at all to the general appearance of the association. Annuals are of very minor importance. The small shrubs of the family Compositæ which occur here and there are too few in number of individuals and are too much like the shadscale in habit of growth and dullness of color to influence materially the aspect of the vegetation. *Kochia vestita* is associated with the shadscale in extensive areas where the vegetation appears otherwise typical of the present association. The much smaller size of the *Kochia* plants makes them inconspicuous.

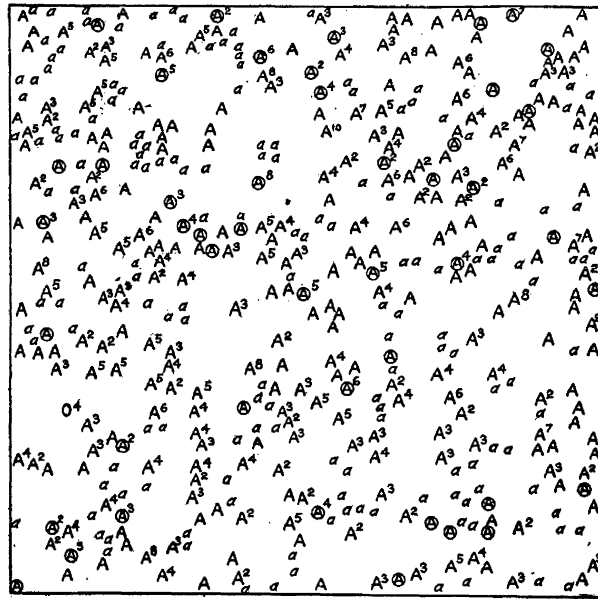


FIG. 8.—A representative 10-meter quadrat of the shadscale association, showing the location of each individual plant of *Atriplex confertifolia* (A), the only woody species present, and of *Opuntia* sp. (O). The figures show the number of main stems and, hence, indicate the size of the plant. A circle around the letter indicates that the plant is dead. Seedlings of *Atriplex* are indicated by the small a. The annual grass *Bromus* (not indicated on the quadrat) was very abundant around the *Atriplex* bushes.

## PHYSICAL CONDITIONS INDICATED

The conditions in shadscale land as regards moisture and salt content of the soil are shown in Table X, which gives the results of various borings in typical areas.

TABLE X.—*Shadscale association: Moisture conditions and salt content of the soil in typical areas.*<sup>1</sup>

Item.	Depth of soil (foot).	Date of collection.								Average.	
		June.				July.	August.				
		3	7	26	26	5	5	6	6		
No. of sample. ....	.....	17	34	54	55	56	72	108	117		
Moisture equivalent.....	{	1	.....	24.2	23.4	21.7	22.4	.....	.....	22.9	
		2	.....	31.4	28.8	36.1	27.9	.....	.....	31.0	
		3	.....	34.0	32.4	35.8	35.8	.....	.....	34.5	
		4	.....	33.7	32.6	29.5	26.9	.....	.....	30.6	
Wilting coefficient.....	{	1	.....	13.1	12.7	11.8	12.2	.....	.....	12.4	
		2	.....	17.0	15.6	19.6	15.1	.....	.....	16.8	
		3	.....	18.5	17.6	19.5	19.5	.....	.....	18.7	
		4	.....	18.3	17.7	16.0	14.6	.....	.....	16.6	
Moisture content above or below wilting coefficient.....	{	1	.....	-6.5	-5.9	-4.7	-5.2	.....	.....	-5.6	
		2	.....	-4.5	-5.2	-5.2	-5.8	.....	.....	-5.2	
		3	.....	-5.1	-4.8	-5.2	-4.8	.....	.....	-5.0	
		4	.....	-4.6	-3.6	-6.6	-7.1	.....	.....	-5.5	
Salt content.....	{	1	0.10	0.05	.05	.07	.06	.06	0.05	0.12	.07
		2	.36	.05	.44	.29	.22	.27	.42	.54	.32
		3	.40	.22	.88	.82	.88	1.06	1.14	.88	.78
		4	.....	.....	.88	.94	.88	.80	.....	1.14	.93

<sup>1</sup> All data in this table are stated in percentages of the dry weight of the soil. The moisture contents with a plus sign (+) represent moisture available for growth (above the wilting coefficient), while those with a minus sign (−) represent a corresponding deficit of available moisture (below the wilting coefficient).

Comparison with the corresponding data in Table IX shows little difference in the physical conditions of the shadscale and Kochia land. The surface foot of soil in the shadscale association usually contains more gravel and is of somewhat lighter texture, as indicated by the somewhat lower average moisture equivalent. This, together with the rougher surface of the land, would indicate more favorable conditions for the penetration of water.<sup>1</sup> On the other hand, the second, third, and fourth feet show a more constant and more pronounced deficit of available moisture than is the case in Kochia land. At first glance this would seem to confute the assumption that the conditions for the penetration of water are better than on Kochia land, but it must be remembered that in shadscale land, which supports much the heavier vegetation, the loss of water by transpiration must be greater.

The average salt content at all depths down to 4 feet is somewhat smaller in shadscale than in Kochia land, but in this respect the difference between the two types is of small importance.

<sup>1</sup> In some parts of the shadscale area, especially where *Kochia vestita* is abundant, a tendency to the formation of hardpan at a depth of about 24 inches was noted, but this condition appears to be exceptional in Tooele Valley.

An 8-foot boring made in a portion of the shadscale area where *Kochia vestita* was also abundant gave interesting results as regards the salinity of the soil at greater depths than were reached by any of the borings included in Tables IX and X. The percentage of salt contents at the successive 1-foot depths were 0.10, 0.79, 1.02, 0.98, 0.92, 0.88, 0.94, and 1.02, which indicates a very uniform condition as regards salt content of the soil below the second foot and to an unknown depth.

The differences in the averages for each physical factor as given in Tables IX and X scarcely seem of sufficient magnitude to explain the separate occurrence of *Atriplex confertifolia* and *Kochia vestita* in distinct associations alternating over large areas, especially when we note that some of the borings in typical portions of each association show almost identical physical conditions. It is not surprising therefore that the line of contact between the two associations is a vague one and that the two species mingle on equal terms over areas of considerable extent. Yet there is a possible explanation for the alternation of these two types which is not brought out by the data given in these tables. In *Kochia* land, because of the less favorable conditions for penetration, the seasonal total of available moisture may not be sufficient to support a stand of shadscale in competition with *Kochia*.

The distribution of *Atriplex confertifolia* appears to be limited toward the upper end of the valley by the occurrence of light, easily permeable soil which is free from an excess of salts to a depth of 3 feet or more. In such land shadscale can not compete with sagebrush. Toward Great Salt Lake it is confined to the drier, better drained land of the hummocks and ridges and is excluded from the flats where the soil is excessively saline and is wet to the surface during much of the year.

Areas of very limited extent are found here and there in which the shadscale plants are much larger than the average and have a green, thrifty appearance, with a notable absence of dead wood. In such spots—generally obvious depressions—the soil conditions are more favorable than in most of the shadscale area, the salt content being lower and the moisture content higher. The results of a boring in one such spot, made on July 13, are given in Table XI.

TABLE XI.—Salt content and moisture conditions of the soil in a spot where *Atriplex confertifolia* was exceptionally large and healthy.<sup>1</sup>

Depth (feet).	Salt content.	Wilting coefficient.	Moisture content above or below wilting coefficient.
1	0.06	20.5	—10.1
2	.05	17.4	— 2.0
3	.09	18.3	+ 2.8
4	.09	18.1	0.0

<sup>1</sup> All data in percentages of the dry weight of the soil.

The low salt content throughout the 4 feet, the relatively high wilting coefficients, and the presence so late in the summer of available moisture in the third foot are worthy of note.

**SUMMARY OF PHYSICAL CONDITIONS.**—The presence of the typical shadscale association as it occurs in Tooele Valley indicates usually (1) a soil of finer texture, having a higher moisture equivalent than in sagebrush land; (2) a deficit in midsummer of moisture available for plant growth; (3) a high salt content of the soil below the depth of 1 or 2 feet;<sup>1</sup> and (4) as compared with land occupied by the *Kochia* associa-

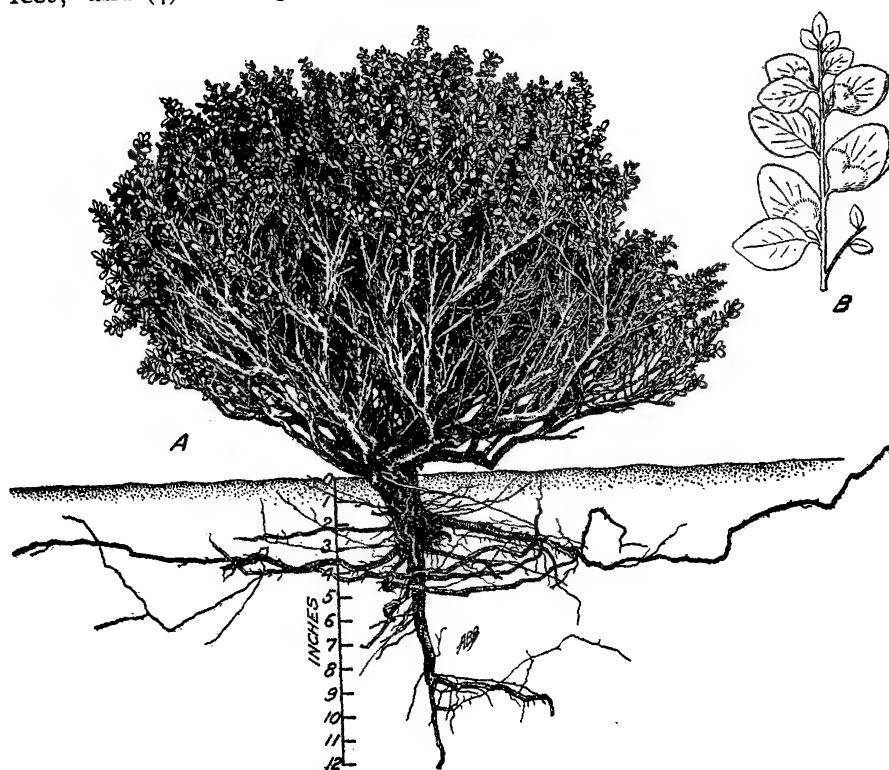


FIG. 9.—*Atriplex confertifolia* (shadscale): A, A typical plant, showing the thick, vertical taproot and the widespreading lateral roots; B, detail of a fruiting branch, showing the shape of the leaves and of the bracts or scales, which envelop the fruits.

tion, a somewhat lighter and more gravelly texture in the first foot and a much more uneven surface—conditions which probably result in better penetration and, hence, in a larger seasonal total of water available for plant growth than on *Kochia* land.

#### ADAPTATIONS TO PHYSICAL CONDITIONS

The dominant species, *Atriplex confertifolia*, need alone be considered in this connection. As shown in figure 9, this plant has a well-developed

<sup>1</sup> In localities outside of Tooele Valley shadscale is not confined to areas having a saline subsoil, but is also found on dry, gravelly soils where the moisture conditions are apparently too extreme for sagebrush.

taproot, its root system being, therefore, very different from that of *Kochia vestita*.

The roots of shadscale, although by no means so deep as those of *Artemisia tridentata*, doubtless as a rule penetrate and obtain water from a greater depth of soil than do the roots of *Kochia*.<sup>1</sup> This would help to explain the fact that, notwithstanding the more favorable conditions for penetration of water, the deficit during periods of drought of moisture available for growth in the second, third, and fourth feet is normally greater in shadscale than in *Kochia* land.

The moribund appearance in 1912 of the shadscale plants in most of the area covered by this association in Tooele Valley points to the conclusion that in years of not more than average rainfall the moisture supply is inadequate. Thus, in 1912 the moisture available for growth had been exhausted to a depth of 4 feet, and the plants had begun to shed their leaves before the end of June. Apparently, with the normal thickness of stand in this association, the older individual plants can not obtain sufficient water to maintain life in all parts of their bodies. The branches are in active competition and the plant as a whole remains alive only by sacrificing some of its members. The death of some of the branches in almost every plant which has passed the seedling stage reduces by so much the transpiring surface and results in a proportionate economy of the scanty moisture available to each individual. To an even greater degree than *Artemisia tridentata* this plant has the faculty of remaining during a great part of the year in a nearly dormant condition, while retaining some of its foliage.

#### EFFECTS OF DISTURBING FACTORS: SUCCESSIONS

The exact stages in the revegetation of shadscale land from which the original plant cover has been removed by fire or by the plow remain to be worked out. There is evidence, however, that *Gutierrezia sarothrae* forms an important stage in these successions. A large area near the center of Tooele Valley is covered with an almost pure growth of this small, yellow-flowered plant of the Compositæ. While a part of this tract was probably once occupied by sagebrush, the greater portion occurs in the midst of the shadscale belt and has a strongly saline subsoil.<sup>2</sup>

<sup>1</sup> Nevertheless, the *Atriplex* roots do not develop well in a strongly saline subsoil. Thus, at a boring where the first foot of the soil contained 0.1 and the second 0.8 per cent of salts, few living roots were found below the depth of 12 inches.

<sup>2</sup> This plant shows marked adaptability to varying soil conditions. In areas having a saline subsoil, which were presumably covered originally with shadscale, the plants of *Gutierrezia* are scattered and small and have a superficial root system, while in nonsaline areas, where sagebrush was probably the original vegetation, the stand is denser, the plants are larger, and a good taproot is developed.

## VARIATIONS FROM THE TYPICAL ASSOCIATION

The shadscale area in Tooele Valley comes into contact on its upper limit with the sagebrush and the *Kochia* associations and on its lower limit with the greasewood-shadscale association. In each case mixed or transitional communities result. The conditions under which shadscale mingles with sagebrush and with *Kochia* have been discussed on preceding pages. The transition to the greasewood-shadscale association, which is a very gradual one, will be treated in connection with the latter association.

## GREASEWOOD-SHADSCALE ASSOCIATION

## TOPOGRAPHICAL RELATIONS

The area occupied by the greasewood-shadscale association forms an interrupted belt (see map, Pl. XLII) across the valley between the areas occupied by the shadscale association, and by the grass flats, respectively. It also covers the low ridges and hummocks which alternate with the basinlike depressions and flats near the shore of Great Salt Lake (Pl. XLIII, fig. 1, and Pl. XLVII, fig. 3). In general, it occupies all areas where the water table is sufficiently high to make moist soil accessible to the deep-rooting greasewood and where at the same time 1 or 2 feet of the surface soil are sufficiently dry to permit the growth of shadscales. Where the water table is too low this association gives place to the pure shadscale. On the other hand, as the soil becomes wet nearer and nearer the surface, the shadscale gradually disappears and at the edge of the grass flats greasewood associates with *Allenrolfea occidentalis* and *Suaeda moquinii* instead of with *Atriplex confertifolia*.

## BOTANICAL COMPOSITION

This type of vegetation is dominated by two shrubby species, greasewood (*Sarcobatus vermiculatus*; see fig. 10, p. 404) and shadscale (*Atriplex confertifolia*; fig. 9, p. 398). In typical areas these plants intermingle in approximately equal numbers, but on the highest ground (Pl. XLVII, fig. 2) shadscale is strongly predominant, while on the lowest land where this association occurs greasewood is the more abundant. *Kochia vestita* is abundant in much of the area occupied by this association, but the plants are so small in comparison with the two dominant species that they do not affect the general appearance of the vegetation. In spots of limited size greasewood and *Kochia* are associated, shadscale being absent. The soil conditions in such spots do not differ materially from those of the typical greasewood-shadscale association. Few other species are found, and of these the number of individuals is usually small. The following list includes all species noted as occurring in the greasewood-shadscale association:



## PERENNIAL SPECIES

<i>Elymus condensatus</i> Presl	<i>Sarcobatus vermiculatus</i> (Hook.) Torr.
<i>Poa</i> sp. ( <i>P. sandbergii</i> Vasey?).	<i>Suaeda moquinii</i> (Torr.) A. Nels.
<i>Sitanion minus</i> Smith	<i>Suaeda intermedia</i> Wats.
<i>Atriplex confertifolia</i> (Torr.) Wats.	<i>Lappula occidentalis</i> (Wats.) Greene
<i>Atriplex nuttallii</i> Wats.	<i>Gutierrezia sarothrae</i> (Pursh) B. and R.
<i>Kochia vestita</i> (Wats.) A. Nels.	<i>Tetradymia nuttallii</i> T. and G.

## ANNUAL AND BIENNIAL SPECIES

<i>Bromus tectorum</i> L.	<i>Sophia pinnata</i> (Walt.) Howell
<i>Erysimum asperum</i> (Greene) Rydb.	<i>Machaeranthera canescens</i> (Nutt.) Gray

The local distribution of most of these plants varies greatly within the area occupied by the association, probably because of the great diversity in the depth to permanent moisture and at which the subsoil becomes strongly saline.

## APPEARANCE

This type of vegetation is less monotonous in its appearance than the sagebrush, *Kochia*, and shadscale associations, owing to the strong contrast in color and usually in size between the two dominant species. (Pl. XLVII, fig. 2.) Greasewood has a bright-green color, changing to yellowish later in the season, and appears dark when photographed against the sun. Shadscale, on the other hand, has a dull brownish gray hue. The former plant often reaches a height of 4 or 5 feet, while the latter seldom exceeds 2 feet.

At the highest elevations occupied by this association there is sufficient moisture for the growth of greasewood only along drainage channels, and the general surface of the land is covered with pure shadscale. Somewhat farther toward Great Salt Lake plants of greasewood are scattered among the shadscale, although much less numerous than the latter. Finally near the borders of the grass flats and on the ridges and hillocks which intersect the salt flats the two species grow side by side on more or less equal terms, and their colors blend when the vegetation is viewed from a short distance.

## PHYSICAL CONDITIONS INDICATED

The soil moisture and salinity conditions, which characterize typical portions of the land occupied by this association, are indicated by the data in Table XII. Comparison with Table X will bring out the differences between this environment and that of the pure shadscale association.

TABLE XII.—*Greasewood-shadscale association: Moisture conditions and salt content of the soil in typical areas.*<sup>1</sup>

Item.	Depth of soil (feet).	Date of collection.							
		June.			July.				Average.
		4	7	27	3	3	6	29	
No. of sample.....		19	35	63	66	69	75	98	
Moisture equivalent.....	1	22.0	.....	26.5	26.5	26.1	28.5	15.5	24.1
	2	22.9	.....	30.8	34.2	32.2	24.8	14.7	26.6
	3	13.3	.....	35.3	31.5	33.5	20.8	22.2	26.1
	4	27.0	.....	30.2	31.5	31.3	17.5	24.2	26.9
Wilting coefficient.....	1	11.9	.....	14.4	14.4	14.2	15.5	8.4	13.1
	2	12.4	.....	16.7	18.6	17.5	13.5	8.0	14.4
	3	7.2	.....	19.2	17.1	18.2	11.2	12.1	14.2
	4	14.7	.....	16.4	17.1	17.0	9.5	13.1	14.6
Moisture content above or below wilting coefficient.....	1	— .1	.....	— .5	— 4.4	— 4.4	— .3	— 4.7	— 2.4
	2	+ 4.5	.....	+ 6.1	+ 4.1	+ 3.2	+ 3.1	+ .4	+ 3.6
	3	+ 3.5	.....	+ 10.2	+ 4.1	+ 4.3	+ 8.8	+ 3.3	+ 5.7
	4	+ 1.0	.....	+ 8.4	+ 3.9	+ 2.8	+ 13.1	+ 2.5	+ 5.3
Salt content.....	1	.08	.05	.64	.27	.54	.61	.23	.34
	2	.61	.38	1.24	.54	1.10	.61	.60	.72
	3	.62	.82	1.85	.65	1.36	.68	1.25	1.03
	4	1.30	.94	1.36	1.05	1.48	.76	.....	1.15
	5	1.96	1.20	.....	.....	.....	.....	.....	1.58

<sup>1</sup> All data in this table are stated in percentages of the dry weight of the soil. The moisture contents with a plus sign (+) represent moisture available for growth (above the wilting coefficient), while those with a minus sign (—) represent a corresponding deficit of available moisture (below the wilting coefficient).

**SOIL MOISTURE.**—The moisture-holding capacity of the soil, as indicated by the moisture equivalent, is somewhat higher in the first foot, but is lower in the second, third, and fourth feet than in the shadscale association. It is significant that moisture available for the growth of plants was present in considerable quantity during the months of June and July in all but the surface foot in the greasewood-shadscale association, while in the shadscale association during the same months there was a marked deficit of available water to a depth of 4 feet. The relatively high moisture content is correlated with the relatively slight elevation above the level of water in the lake and with a consequently high ground-water table.

**SALINITY.**—The average salt content of each of the first 4 feet of the soil is much higher than in the shadscale association, the difference being especially marked in the second foot, which contains, on the average, as much salts as does the third foot in land occupied by pure shadscale.

**SUMMARY OF PHYSICAL CONDITIONS.**—In Tooele Valley the presence of typical greasewood-shadscale vegetation indicates soil conditions as follows: (1) A fairly high moisture equivalent; (2) the surface foot well drained and usually dry during the summer; (3) moisture available for the growth of plants present throughout the summer at a comparatively slight depth; (4) a high salt content from the second foot downward and often in the surface foot as well.

## ADAPTATIONS TO THE PHYSICAL CONDITIONS

The two dominant species have somewhat different soil requirements, and the land occupied by this association offers a combination of conditions which permits them to grow side by side. Greasewood prefers an ample and permanent supply of moisture within reach of its roots, and its strong, deeply penetrating taproot (fig. 10) enables it to reach moisture in places where the surface soil is dry and the ground-water table is at a considerable depth. This plant can live in soil which is moist to the surface, although under such conditions the plants are never as large and vigorous as where a higher elevation and a subsoil of light texture afford better drainage. Shadscale, on the other hand, does not thrive with its roots in wet soil, and its presence is usually a reliable indication that at least the surface foot is dry during the greater part of the summer.

Greasewood (*Sarcobatus vermiculatus*) grows in a greater variety of habitats than any other flowering plant of the Tooele Valley. It was found in one place or another in company with the dominant species of all of the leading associations. In much the greater part of its range in the valley greasewood is associated with shadscale, but there are exceptions to this rule. The largest and thriftiest looking greasewood plants<sup>1</sup> grew on the summits of dunes of pure sand, together with sagebrush, juniper, *Eriocoma*, *Abronia*, *Eriogonum*, *Psoralea*, and other characteristic plants of the sand-hill mixed association. Shadscale is absent from this community. At the other extreme greasewood occurs in company with *Allenrolfea* in land which is too wet and saline for the growth of shadscale. The widely different conditions in these two environments are indicated by the data in Table XIII.

TABLE XIII.—Moisture equivalent and salt content of the soil where *Sarcobatus vermiculatus* occurred—on the sand hills and with *Allenrolfea*.<sup>1</sup>

Depth (feet).	Moisture equivalent.		Salt content.	
	On sand hills.	With <i>Allenrolfea</i> .	On sand hills.	With <i>Allenrolfea</i> .
1	6.2	31.0	0.09	2.16
2	6.8	37.3	.08	2.08
3	6.1	27.7	.14	1.76
4	7.0	25.9	.16	1.25

<sup>1</sup> All data in percentages of the dry weight of the soil.

The growth of greasewood on the sand hills makes it evident that this plant is not an infallible alkali indicator, although in the great majority of cases its occurrence is associated with an excess of salts in the soil, and in its ability to endure the presence of alkali it is surpassed by few other

<sup>1</sup> The individual alongside the boring made in the sand hills (see Table XIII) was 6 feet high, 10 feet across, and had several stems which were from 1 to 2 inches in diameter at the surface of the ground.

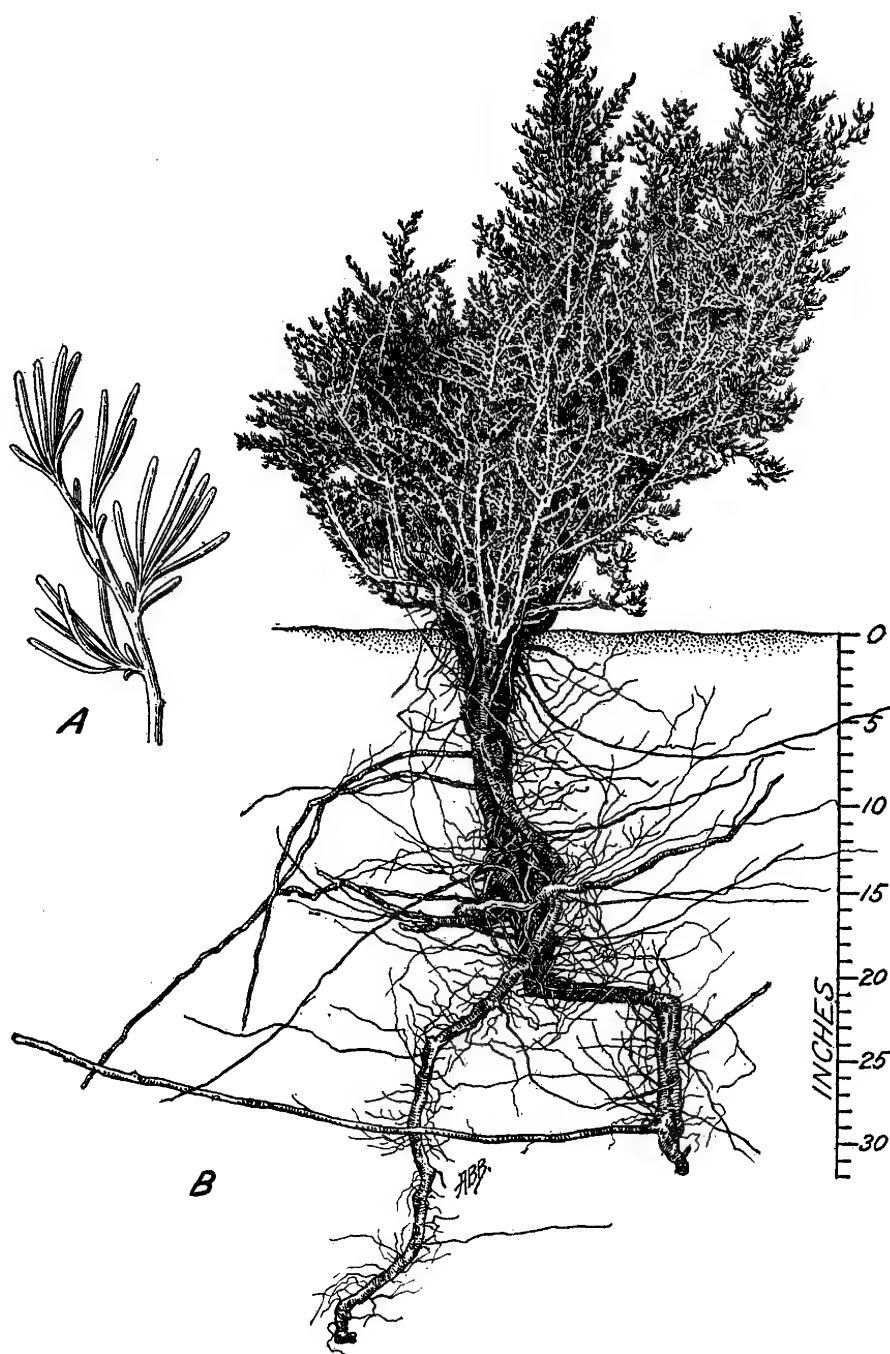


FIG. 10.—*Sarcobatus vermiculatus* (greasewood): A, Detail showing the narrow, rather fleshy leaves; B, a plant showing the excellent root development. The large, deeply penetrating taproot is characteristic of this species.

flowering plants.<sup>1</sup> A condition which is almost always correlated with the presence of greasewood is a permanent supply of moisture available for growth within the depth of soil penetrated by its roots.

#### GRASS-FLAT COMMUNITIES<sup>2</sup>

##### TOPOGRAPHICAL RELATIONS

The grass-flat of vegetation occurs in an interrupted belt (see map, Pl. XLII), which crosses the northern part of the valley and lies between the area occupied by the main body of the greasewood-shadscale association and the salt flats. It covers a gently sloping or nearly level expanse and appears to be lower in elevation than some of the ridges and hillocks situated between it and the shore of the lake. The area is thus somewhat analogous to a coastal lagoon and may have had a similar origin. It is characterized during the greater part of the year by a very moist condition of the soil, due probably in part to seepage.

##### APPEARANCE AND BOTANICAL COMPOSITION

The vegetation of the grass flats shows considerable diversity. Several plant communities can be distinguished, although the boundaries are rarely very sharp. The two most important of these are characterized by the dominance of (1) tussock grass, or purple top (*Sporobolus airoides*), and rabbit brush (*Chrysothamnus graveolens glabrata*), and (2) salt grass (*Distichlis spicata*). The rabbit brush is also frequently associated with greasewood (*Sarcobatus vermiculatus*), especially along lines of contact between greasewood-shadscale areas and the grass flats. For the most part the vegetation of the grass flats is distinctly halophytic in character, but in limited areas around springs and flowing wells it resembles that of an ordinary nonsaline meadow.

A list of the species which were noted as composing the grass-flat vegetation follows:

##### PERENNIAL SPECIES

<i>Triglochin maritima</i> L.	<i>Halimolobos cymbalaria</i> (Pursh) Greene
<i>Triglochin palustris</i> L.	<i>Dodecatheon</i> sp.
<i>Distichlis spicata</i> (L.) Greene	<i>Glauca maritima</i> L.
<i>Poa nevadensis</i> Scribner	<i>Aster pauciflorus</i> Nutt.
<i>Puccinellia airoides</i> (Nutt.) Wats.	<i>Chrysothamnus graveolens glabrata</i> (Gray)
<i>Spartina gracilis</i> Trin.	A. Nels.
<i>Sporobolus airoides</i> Torr.	<i>Crepis glauca</i> (Nutt.) T. and G.
<i>Juncus balticus</i> Willd.	<i>Iva axillaris</i> Pursh
<i>Iris</i> sp. (probably <i>I. missouriensis</i> Nutt.).	

<sup>1</sup> At Grand Junction, Colo., young seedlings of greasewood were found growing where the soil to a depth of 2 inches, which was about the limit to which their roots had penetrated, gave a specific resistance of 36 ohms, indicating the presence of at least 2.5 per cent of salts.

<sup>2</sup> The ecological status of the vegetation of the grass flats can not be determined until further investigations in the Great Basin region shall have been made. For the present, therefore, it seems advisable to use the general term "community" in referring to these types.

## ANNUAL AND BIENNIAL SPECIES

*Hordeum jubatum* L.  
*Salicornia rubra* A. Nels.  
*Suaeda erecta* (Wats.) A. Nels.  
*Atriplex spatiosa* A. Nels.  
*Cleome serrulata* Pursh

*Melilotus alba* Desv.  
*Erythraea arizonica* (Gray) Rydb.  
*Orthocarpus tolmiei* H. and A.  
*Carduus scariosus* (Nutt.) Heller

## PHYSICAL CONDITIONS INDICATED

Reference to Tables XIV and XV shows that there is much variation in the moisture and salinity conditions of the grass-flat area, but, broadly speaking, the soils are characterized by (1) a high moisture-holding capacity, ascribable partly to the fine texture and partly to the large quantity of organic matter present, (2) the presence near the surface and usually throughout the summer of moisture available for growth (above the wilting coefficient), and (3) a salt content sufficiently high to be injurious to many crop plants. The soils under the salt-grass community (Table XV), while usually much more saline than under the *Sporobolus-Chrysothamnus* community (Table XIV), have an average salinity inferior to that of the salt flats (Tables XVI and XVII).

## SPOROBOLUS-CHRYSOTHAMNUS COMMUNITY

The *Sporobolus-Chrysothamnus* community (Pl. XLVIII, fig. 3) covers a large part of the grass-flat area in Tooele Valley. In places one or the other of the dominant species occurs where the other is absent, but they are more often closely associated. Salt grass (*Distichlis spicata*) is also usually more or less abundant in this community.

Tussock grass (*Sporobolus airoides*) forms coarse mats, which are as a rule closely grazed by animals. In late summer the feathery purple panicles of this grass are a characteristic feature of the vegetation of the grass flats in such areas as are not grazed. The rabbit brush (*Chrysothamnus graveolens glabrata*) is a much-branched shrub, from 2 to 4 feet high, with whiplike slender branches having green bark and very small, narrow leaves. Its color is bright green, modified in late summer by the numerous small heads of yellow flowers which resemble those of goldenrod. The physical conditions where this community occurs are indicated by the data given in Table XIV.

TABLE XIV.—*Sporobolus-Chrysothamnus* community: Salt content and moisture conditions of the soil in typical areas.<sup>1</sup>

Item.	Depth of soil (feet).	Date of collection.										Aver- age.
		June 4.	July 29.	August 26.								
No. of sample.....		18 SC.	100 C.	S.	S.	S.	SC.	SC.	SC.	C.	C.	
Salt content.....	{ 1	0.22	0.25	0.85	0.45	0.35	0.20	0.20	0.25	0.35	0.20	0.33
	2	.46	.45	.40	.40	.60	.25	.35	.45	.65	.45	.44
	3	.53	.58	.20	.20	.50	.20	.30	.15	1.05	.35	.40
	4	.26	.30	.20			.15	.30	.15			.23
	5	.14										
Moisture equivalent....	{ 1	34.0	29.5									
	2	29.1	35.3									
	3	34.3	46.6									
	4	26.4	36.7									
	5	31.6										
Wilting coefficient.....	{ 1	18.5	16.0									
	2	15.8	19.2									
	3	18.6	25.3									
	4	14.3	19.9									
	5	17.1										
Moisture content above or below wilting co- efficient.....	{ 1	-0.8	+4.5									
	2	+4.4	+11.4									
	3	+5.8	+25.6									
	4	+9.8	+14.3									
	5	+6.1										

<sup>1</sup> All data in this table are stated in percentages of the dry weight of the soil. The moisture contents with a plus sign (+) represent moisture available for growth (above the wilting coefficient), while those with a minus sign (-) represent a corresponding deficit of available moisture (below the wilting coefficient). The unnumbered borings were made on Aug. 26, 1913, and the letters indicate whether the vegetation was dominated by *Sporobolus* without *Chrysothamnus* (S), *Sporobolus* with *Chrysothamnus* (SC), or *Chrysothamnus* without *Sporobolus* (C).

SALT-GRASS (*DISTICHLIS*) COMMUNITY

*Distichlis spicata*, well known as salt grass throughout the western United States, is a low-growing, harsh-leaved grass which spreads by creeping rootstocks. It tends to form a heavy sod, especially where the land is grazed, and under such conditions this plant is very efficient in adding humus to the soil.

Salt grass is more or less abundant in all parts of the grass flats and also penetrates the salt flats (Pl. XLVII, fig. 3), where in some places it associates scatteringly with *Allenrolfea* and in other places forms dense mats. In the wetter portions of the grass flats salt grass is the principal component of a meadowlike vegetation, with *Juncus balticus*, *Suaeda erecta*, *Puccinellia airoides*, and *Glaux maritima* as important associates and with numerous other species occasionally present.

The conditions as regards soil moisture and salinity at borings where this community occurs are stated in Table XV.

TABLE XV.—*Salt-grass community: Moisture conditions and salt content of the soil in typical areas.*<sup>1</sup>

Item.	Depth of soil (feet).	Date of collection.						Average.
		June.	July.				August.	
		4	6	12	12	29	6	
No. of sample.....		20	73	83	84	101	109	
Moisture equivalent.....	1	28.5	28.4	.....	30.9	48.9	.....	34.1
	2	32.6	17.1	.....	22.6	54.9	.....	31.8
	3	35.1	19.4	.....	13.2	65.8	.....	33.3
	4	.....	36.1	.....	.....	62.2	.....	49.1
Wilting coefficient.....	1	15.5	15.4	.....	16.5	26.6	.....	18.5
	2	17.7	9.3	.....	12.3	29.6	.....	17.3
	3	19.1	10.5	.....	7.2	35.8	.....	18.1
	4	.....	19.6	.....	.....	33.8	.....	26.7
Moisture content above or below wilting coefficient.....	1	+ 3.1	+ 8.9	.....	.....	+16.7	.....	+ 9.6
	2	+ 9.5	+ 6.9	.....	.....	+24.2	.....	+13.5
	3	+11.5	- 1.3	.....	.....	+24.2	.....	+11.4
	4	.....	- 4.5	.....	.....	+32.2	.....	+13.8
Salt content.....	1	.25	2.30	0.57	.53	.59	2.18	1.07
	2	.56	1.64	.80	.24	.72	1.85	.97
	3	.76	1.36	.60	.....	.34	.....	.76
	4	1.02	1.14	.24	.....	.18	.....	.64

<sup>1</sup> All data in this table are stated in percentages of the dry weight of the soil. The moisture contents with a plus sign (+) represent moisture available for growth (above the wilting coefficient), while those with a minus sign (—) represent a corresponding deficit of available moisture (below the wilting coefficient).

SALT-FLAT COMMUNITIES<sup>1</sup>

## TOPOGRAPHICAL RELATIONS

Along the margin of Great Salt Lake there is a belt of low land which varies in width from about 4 miles, near the axis of the valley, to a mere fringe on the east and west sides where the mountain ranges approach the lake shore. Much of this area (see map, Pl. XLII) is covered with water at times, but in summer presents a dazzling white surface due to the heavy crust of salts (Pl. XLIII, fig. 1, and Pl. XLVIII, fig. 1). These flats are divided into shallow basins of greater or less extent, separated by low ridges and hummocks. (See Pl. XLII, detail of vegetation west of Grants.) All but the lowest of these elevations are occupied by the greasewood-shadscale association (see foreground of Pl. XLIII, fig. 1), while the basins and flats when not altogether devoid of vegetation support a few extremely halophytic species (Pl. XLVIII, figs. 1 and 2), which occur either as scattered individuals or in crowded colonies.

The two environments are ecologically quite distinct, but it is impracticable to indicate on a map of the small scale used in Plate XLII the areas actually occupied by elevations and by depressions, with their respective types of vegetation. Greasewood occurs not only on the higher ridges in association with shadscale, but also on the lower hummocks and at the edges of the depressions, in association with *Allenrolfea*. Shadscale, on the other hand, is not found in the depressions, nor do the typical salt-flat species occur on the higher ridges.

<sup>1</sup> The ecological status of the salt-flat vegetation, like that of the grass-flat vegetation, can not be determined without more extensive investigation in the Great Basin region. In the present paper it seems advisable to use the general term "community" in referring to these types.



The vegetation of the flats and depressions comprises several communities, each characterized by the presence of a single species—*Allenrolfea occidentalis*, *Salicornia utahensis*, and *S. rubra*. The first of these is by far the most abundant and widely distributed. These three species appear to be the most salt resistant of the flowering plants of this region, taking possession of the land left bare by the recession of the lake as soon as its salt content has been reduced sufficiently from the point of saturation with the excessively saline lake water to permit the growth of any flowering plant.

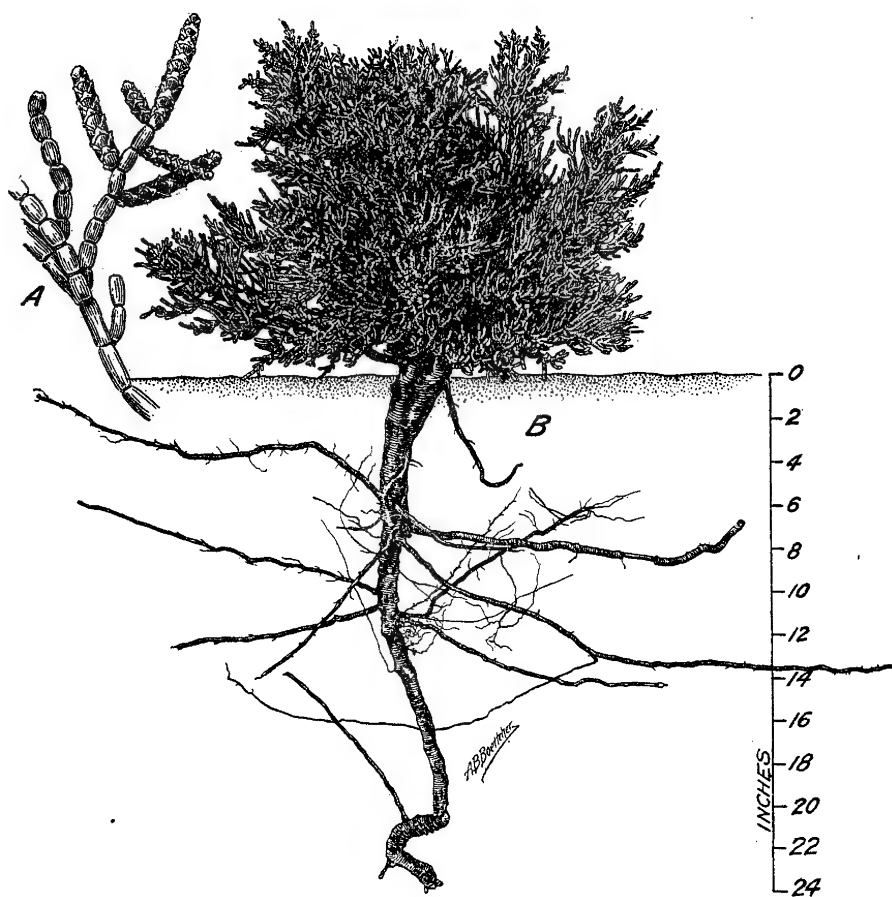


FIG. 11.—*Allenrolfea occidentalis*: A, Detail of a fruiting branch, showing the cylindrical, fleshy, practically leafless stems; B, a plant showing the large taproot and rather scanty lateral roots characteristic of this species.

#### ALLENROLFEA COMMUNITY

APPEARANCE AND BOTANICAL COMPOSITION.—The dominant species, *Allenrolfea occidentalis*, is a shrubby plant with numerous cylindrical, jointed, fleshy, practically leafless branches and a large taproot (fig. 11). In Tooele Valley it rarely exceeds a height of 2 feet. There is considerable variation in the habitat of this plant, but it develops most characteristically on low hummocks on the salt flats (Pl. XLVIII, fig. 1) and near the bases of the higher ridges, usually preferring a slightly

better drained and less saline soil than the species of *Salicornia*. In places, however, it is seen scattered over the surface of the flats, the

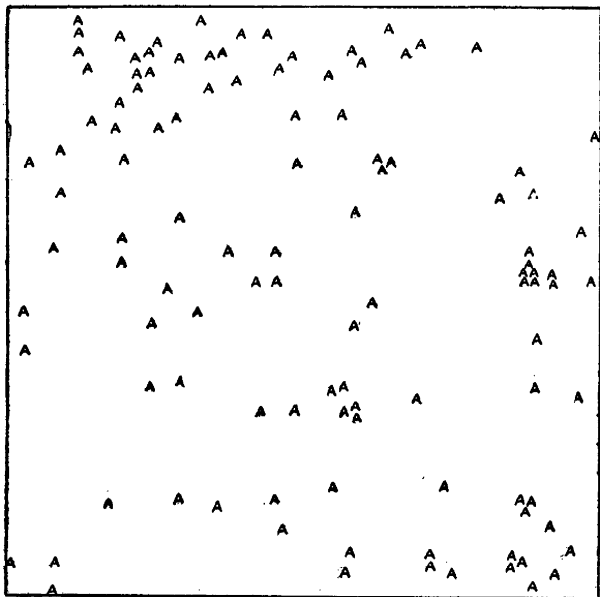


FIG. 12.—A representative 10-meter quadrat of the *Allenrolfea* community (salt-flat association), showing the location of each individual plant of *Allenrolfea occidentalis*, the only species present.

dark brownish green tufts of *Allenrolfea* contrasting strikingly with the pure white of the saline incrustation. The thinness of the stand is shown in figure 12, which represents a typical 10-meter quadrat. Often, as shown in Plate XLVIII, figure 1, *Allenrolfea* forms a pure community. On higher and better drained ground, however, it is frequently associated with *Sarcobatus vermiculatus* and with *Suaeda moquinii*, while in the wetter depressions

it often mingles with *Salicornia utahensis*. Plants of greasewood, when growing with *Allenrolfea*, are usually stunted and sickly looking.

PHYSICAL CONDITIONS INDICATED.—The conditions as to soil moisture and salt content at all borings where *Allenrolfea* occurred are given in Table XVI.

TABLE XVI.—*Allenrolfea* community: Moisture conditions and salt content of the soil in typical areas.<sup>1</sup>

Item.	Depth of soil (feet).	Date of collection.									Average.
		June.		July.						Aug.	
		4	27	6	6	12	12	12	12	29	6
No. of sample.....		21	64	73	76	80	81	82	84	97	109
Moisture equivalent.	1	31.1	28.4	25.5	24.4	.....	.....	26.7	24.6	.....	26.7
	2	37.4	17.1	25.0	19.3	.....	.....	13.2	28.2	.....	23.4
	3	27.9	19.4	26.0	17.3	.....	.....	.....	18.5	.....	21.8
	4	26.0	36.1	30.2	.....	.....	.....	.....	29.5	.....	30.4
Wilting coefficient..	1	16.9	15.4	13.9	13.2	.....	.....	14.5	13.4	.....	14.5
	2	20.3	9.3	13.9	10.5	.....	.....	7.2	15.3	.....	12.7
	3	15.1	10.5	14.1	9.4	.....	.....	.....	10.0	.....	11.8
	4	14.1	19.6	16.4	.....	.....	.....	.....	16.0	.....	16.5
Moisture content above or below the wilting coefficient.	1	+ 3.5	+ 8.9	- 2.0	+ 8.0	.....	.....	.....	- 7.2	.....	+ 2.2
	2	+ 12.3	+ 6.9	+ .8	+ 8.6	.....	.....	.....	+ 2.1	.....	+ 6.1
	3	+ 6.9	- 1.3	+ 2.9	+ 9.7	.....	.....	.....	+ 5.5	.....	+ 4.7
	4	+ 4.3	- 4.5	+ 7.3	.....	.....	.....	.....	+ 7.7	.....	+ 3.7
Salt content.....	1	0.43	2.18	2.30	.25	1.36	1.85	1.36	.55	.18	2.18
	2	.82	2.08	1.64	.26	1.24	1.36	.91	.24	.76	1.85
	3	.98	1.64	1.36	.19	2.30	.88	.98	.....	.76	1.13
	4	.....	1.24	1.16	.44	.....	.88	.98	.....	.....	.94

<sup>1</sup> All data in this table are stated in percentages of the dry weight of the soil. The moisture contents with a plus sign (+) represent moisture available for growth (above the wilting coefficient), while those with a minus sign (−) represent a corresponding deficit of available moisture (below the wilting coefficient).

Several of these samples—e. g., Nos. 76 and 97—were taken at places where *Allenrolfea* grew in company with *Sarcobatus* and where the salt content and moisture content of the soil were lower than in the typical *Allenrolfea* community. It is clear, nevertheless, that the presence of this plant is an almost invariable indicator that the soil (1) contains moisture available for growth, at least below the surface foot, throughout the summer; and (2) is excessively saline to a depth of at least 4 feet.

#### SALICORNIA UTAHENSIS COMMUNITY

APPEARANCE AND BOTANICAL COMPOSITION.—*Salicornia utahensis*<sup>1</sup> (Pl. XLVIII, fig. 2) is a nearly leafless plant with fleshy, jointed stems. It resembles small plants of *Allenrolfea*, but is readily distinguished by the light blue-green color and by the fact that the branches are opposite, while in *Allenrolfea* they are alternate. It spreads by creeping rootstocks and forms pure colonies of greater or less size which sometimes cover the bottoms of depressions (see right end of Pl. XLIII, fig. 1), sometimes occupy hummocks elevated but a few inches above the general surface of the flats. In this case the appearance is much like the *Allenrolfea* hummocks (Pl. XLVIII, fig. 1), except that the latter are higher and the plants are larger and darker colored. This *Salicornia* is also found in association with *Allenrolfea* and with *Distichlis*.

PHYSICAL CONDITIONS INDICATED.—No determinations were made of the moisture equivalent and moisture content of the soil where this community occurs, but two borings carried to a depth of 30 inches and 12 inches, respectively, showed that abundant moisture was present throughout that depth, as would be expected from the slight elevation of the land above the water surface of the lake. The salt contents of different depths of the soil from the borings in question are given in Table XVII.

TABLE XVII.—Salt content of soil in the *Salicornia utahensis* community.

Depth of soil.	Salt content.	
	Sample No. 1.	Sample No. 2.
<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>
0 to 6	2. 20	>2. 50
7 to 12	>2. 50	.....
0 to 12	.....	2. 25
13 to 18	>2. 50	.....
18 to 30	2. 20	.....

#### SALICORNIA RUBRA COMMUNITY

This small, shallow-rooted annual species of *Salicornia* is found most abundantly in pure communities along drainage channels in the salt flats. The patches of *Salicornia rubra* are very conspicuous late in the summer,

<sup>1</sup> This species was recently described from specimens collected by the writers in Tooele Valley by Mr. Ivar Tidestrom. (A new *Salicornia*. Proc. Biol. Soc. Wash., v. 26, p. 13, 1913.)

owing to the bright-red color then assumed by the plants. Scattered individuals of this species were also observed far out on the otherwise bare salt flats.

## CORRELATIONS BETWEEN THE TYPES OF VEGETATION AND THE CHARACTER AND PRODUCTIVITY OF THE LAND

### CORRELATIONS WITH PHYSICAL CONDITIONS

The natural vegetation of Tooele Valley consists of a few easily recognizable plant communities, the distribution of which is largely determined by the moisture relations and the salt content of the soil. The areas occupied by each community are rather sharply delimited, although

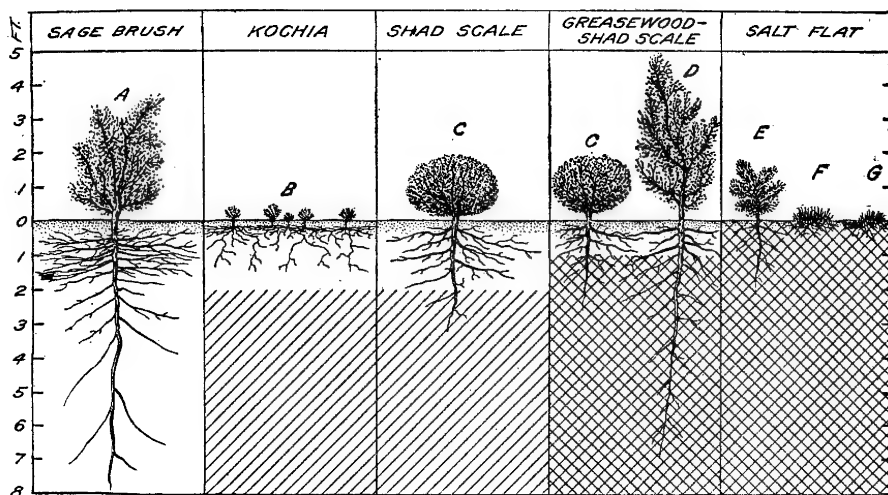


FIG. 13.—Diagram showing the characteristic root development of the dominant species of each of the principal types of vegetation of Tooele Valley, and indicating the average conditions of soil moisture and salinity of the corresponding types of land. The double hatching indicates soil containing an excessive quantity of salt (more than 0.5 per cent) and containing moisture available for growth (above the wilting coefficient) during the summer. The single hatching indicates soil containing more than 0.5 per cent of salts and no moisture available for growth during the summer. No hatching indicates soil containing less than 0.5 per cent of salts and no moisture available for growth during the summer. A, *Artemisia tridentata*; B, *Kochia vestita*; C, *Atriplex confertifolia*; D, *Sarcobatus vermiculatus*; E, *Allenrolfea occidentalis*; F, *Salicornia utahensis*; G, *Distichlis spicata*.

along their boundaries, where the soils are of an intermediate character, the vegetation is more or less mixed. Where, as a result of the removal of the original vegetation by fire or by the plow, secondary plant communities have developed, the correlations between the vegetation and the physical properties of the underlying soils are not always well marked. But with these exceptions, which have been sufficiently discussed on preceding pages, all important variations in the character of the soil are clearly expressed in the appearance and botanical composition of the plant covering. In other words, the principal types of vegetation, where typically developed, are reliable indicators of the physical conditions of the environment. These correlations are stated in Table XVIII, which follows, and are graphically represented in figure 13.

TABLE XVIII.—Principal types of vegetation of Tooele Valley in relation to average soil moisture and salinity conditions.<sup>1</sup>

Plant community.	Moisture and salinity conditions.		
	Source of moisture.	Surface foot of soil.	Soil below surface foot.
Sagebrush.....	Direct precipitation..	Nonsaline, usually dry in summer.	Nonsaline, usually dry in late summer.
S a n d-h i l l mixed.	.....do.....	.....do.....	Nonsaline, usually (?) moist in summer.
Shadscale.....	.....do.....	.....do.....	Saline, usually dry in late summer.
Kochia.....	.....do.....	.....do.....	Saline, usually dry in late summer.
Greasewood-shadscale....	Direct precipitation and high water table.	Saline or nonsaline, usually dry in summer.	Saline, moist.
Grass flat.....	Direct precipitation, high water table, springs and irrigation.	Moderately saline, moist.	Moderately saline, moist.
Salt flat.....	Direct precipitation and high water table.	Saline, moist.....	Saline, moist.

<sup>1</sup> The term "dry" as here applied to the soil indicates that its water content is below the wilting coefficient. The term "moist" implies that moisture available for plant growth (above the wilting coefficient) is present.

The average conditions as respects moisture and salinity of the soil which characterize the land occupied by each of the more important types of vegetation are stated in Table XIX. The data for the different samples upon which these averages are based are given in full under the respective associations (Tables IV, IX, X, XII, XV, and XVI). Only typical areas of each plant community have been taken into account in computing the averages.

TABLE XIX.—Moisture conditions and salt content of the soil in typical areas occupied by the principal plant communities.<sup>1</sup>

Soil depth (feet).	Plant community.					
	Sagebrush ( <i>Artemisia tridentata</i> ).	Kochia ( <i>Kochia vestita</i> ).	Shadscale ( <i>Atriplex confertifolia</i> ).	Greasewood-shadscale ( <i>Sarcobatus</i> and <i>Atriplex</i> ).	Grass flat. Salt grass ( <i>Distichlis spicata</i> ).	Salt flat. <i>Allenrolfea occidentalis</i> .

## MOISTURE EQUIVALENT.

1.....	14.2	25.8	22.9	24.1	34.1	26.7
2.....	15.6	27.0	31.0	26.6	31.8	23.4
3.....	16.5	33.5	34.5	26.1	33.3	21.8
4.....	15.8	31.9	30.6	26.9	49.1	30.4

<sup>1</sup> All data are given as percentages of the dry weight of the soil.

TABLE XIX.—Moisture conditions and salt content of the soil in typical areas occupied by the principal plant communities—Continued.

Soil depth (feet).	Plant community.					
	Sagebrush ( <i>Artemisia tridentata</i> ).	Kochia ( <i>Kochia vestita</i> ).	Shadscale ( <i>Atriplex confertifolia</i> ).	Greasewood-shadscale ( <i>Sarcobatus</i> and <i>Atriplex</i> ).	Grass flat. Salt grass ( <i>Distichlis spicata</i> ).	Salt flat. <i>Allenrolfea occidentalis</i> .
WILTING COEFFICIENT.						
1.....	7.7	14.0	12.4	13.1	18.5	14.5
2.....	8.5	14.7	16.8	14.4	17.3	12.7
3.....	8.9	18.2	18.7	14.2	18.1	11.8
4.....	8.6	17.3	16.6	14.6	26.7	16.5
MOISTURE CONTENT ABOVE OR BELOW WILTING COEFFICIENT.						
1.....	-2.5	-5.4	-5.6	-2.4	+9.6	+2.2
2.....	-.6	-1.8	-5.2	+3.6	+13.5	+6.1
3.....	-1.3	-2.5	-5.0	+5.7	+11.4	+4.7
4.....	+1.0	-2.4	-5.5	+5.3	+13.8	+3.7
Average....	-.8	-3.0	-5.3	+3.0	+12.1	+4.2
SALT CONTENT.						
1.....	0.03	0.12	0.07	0.34	1.07	1.26
2.....	.03	.55	.32	.72	.97	1.11
3.....	.05	1.02	.78	1.03	.76	1.13
4.....	.07	1.11	.93	1.15	.64	.94
Average....	.04	.70	.52	.81	.86	1.11

## CORRELATIONS WITH CROP PRODUCTION.

The capabilities of the principal types of land in Tooele Valley for crop production with or without irrigation are summarized in Table XX.

TABLE XX.—Crop-producing capabilities of the land as indicated by a normal growth of the different types of vegetation.

Type of vegetation.	Is land capable of crop production?	
	Without irrigation.	With irrigation.
Sagebrush.....	Yes.....	Yes.
Kochia.....	Precariously in years of rainfall above the normal.	Yes; if alkali can be removed.
Shadscale.....	Precariously; conditions rather more favorable than on Kochia land.	Yes; after alkali is removed.
Greasewood-shadscale.....	No.....	Yes; after alkali is removed.
Grass flats.....	Probably not.....	Possibly, with drainage.
Salt flats.....	No.....	No.

**SAGEBRUSH LAND.**—This is the only type in Tooele Valley which is well adapted to dry farming. Practically all of the area devoted to wheat in this valley was doubtless originally occupied by the sagebrush association. Most of this area is situated on the eastern side of the valley, where the rainfall is heavier than on the western side. But the presence of sagebrush does not necessarily indicate good conditions for dry farming. Where the stand is thin and the plants are small and unthrifty, the depth of good soil is too slight for profitable crop production without irrigation. Sagebrush vegetation of this character indicates the presence of gravelly hardpan, or else of an excessive quantity of alkali salts, at a depth of only 2 or 3 feet.

A good growth of sagebrush also indicates the best land for farming under irrigation. Because of the low water table and the absence of alkali salts, such land is not likely to require artificial drainage.

**KOCHIA LAND.**—Dry farming is sometimes attempted on Kochia land, rye being the crop which is usually grown. The yields obtained are very small, at least in years of only normal rainfall, the depth of good soil being narrowly limited by the strongly saline subsoil. Whether Kochia land is suitable for irrigation farming is somewhat doubtful, since the rather impervious character of the soil might make it difficult to leach the salts to a sufficient depth to insure profitable crop production.

**SHADSCALE LAND.**—Dry farming is precarious on this type of land. On the other hand, it seems probable that most of the shadscale land in Tooele Valley would produce crops under irrigation, if water for this purpose were available, since as compared with Kochia land the soil is more permeable and there is greater likelihood that the salts could be leached out of the subsoil.

**GREASEWOOD-SHADSCALE LAND.**—One or two attempts at crop production without irrigation on this type of land were observed, but the results seemed to be no better than on Kochia and on shadscale land. The reason doubtless is that while the moisture conditions are more favorable than on the latter types the salt content of the soil at only a slight depth is too high to permit crop plants to make a satisfactory root development.

On the other hand, greasewood-shadscale land when irrigated and reclaimed produces good crops of alfalfa, grain, and even of orchard fruits. Artificial drainage, however, would probably be required in case an extensive area of this type of land were under irrigation, the water table being already high and the subsoil strongly saline.

**GRASS-FLAT LAND.**—This type of land affords pasturage to horses and cattle and is therefore by no means negligible as one of the agricultural resources of the valley. Drainage would probably be necessary in order to fit it for crop production.

**SALT-FLAT LAND.**—Most of the area occupied by this type of vegetation is too wet and too saline for crop production and offers little prospect of successful reclamation.

## SUMMARY

In Tooele Valley the different types of native vegetation indicate the conditions of soil moisture and salinity of the land on which they are found and thus afford a basis for estimating its capabilities for crop production. These correlations are stated in Table XVIII (p. 413), Table XIX (p. 413), and Table XX (p. 414).

The sagebrush (*Artemisia tridentata*) association covers the land nearest the mountains where the soil is of rather light texture, permeable, rather low in moisture-holding capacity, and free from an excess of alkali salts and where under natural conditions the moisture available for growth is usually exhausted early in summer. A good stand and growth of sagebrush indicates land that is well adapted to both dry farming and irrigation farming; but where the stand is thin and the growth poor the depth of good soil is usually too small for profitable crop production, at least without irrigation.

The Kochia (*Kochia vestita*) association covers areas lying just below the sagebrush belt and also occupies islands in the midst of the latter vegetation. The soil, which is remarkably homogeneous, differs from that of sagebrush land in its finer texture, relative impermeability, higher moisture-holding capacity, and the high salt content of the subsoil. The first foot of soil is usually free from an injurious quantity of alkali salts. Moisture available for growth is usually wanting during the summer to a depth of at least 4 feet and probably to a much greater depth. Dry farming is precarious on such land, owing to the small depth of soil free from alkali. Even under irrigation the relatively impervious nature of the soil might hinder washing out the salts to a depth which would permit profitable crop production.

The shadscale (*Atriplex confertifolia*) association occupies the land next below the Kochia belt. The soil is similar, in the main, to that where Kochia occurs, but frequently contains much gravel, is usually even drier during the summer months, and has on the average a somewhat smaller salt content. Dry farming is nearly as precarious on shadscale land as on Kochia land, but where water is available for irrigation the salts could probably be leached to a greater depth than on Kochia land, the soil being more permeable.

The greasewood-shadscale (*Sarcobatus vermiculatus* and *Atriplex confertifolia*) association occupies a belt lying between the pure shadscale vegetation and the salt flats and also crowns the ridges and knolls which intersect the latter. The soil differs from that of any of the foregoing associations in usually containing, during the summer, moisture available for growth at all depths below the surface foot. It is also strongly saline below the depth of 1 foot, and even the surface foot often contains a considerable quantity of salts. Land of this type is not suitable for dry farming, but can be made to produce good crops under irrigation, especially when drainage is provided.



The presence of the grass-flat (*Sporobolus*, *Distichlis*, *Chrysothamnus*) vegetation indicates a soil which has a high moisture capacity, is more or less saline, and is moist to the surface during a great part of the year. Such land produces a coarse natural pasturage, but is not suitable for crop production unless it is drained.

The salt-flat (*Allenrolfea*, *Salicornia*) vegetation occupies land which is extremely saline and is wet to the surface during a great part of the year. This type of land is not adapted to crop production.

The correlations above outlined are yet known to apply only in Tooele Valley. Further investigation is needed in order to establish their applicability in the classification of agricultural land in other parts of the Great Basin.

**PLATE XLII.** Sketch map showing the distribution and relative areas of the different types of vegetation in Tooele Valley, with detail showing depressions covered with salt-flat vegetation alternating with ridges bearing greasewood-shadscale vegetation.

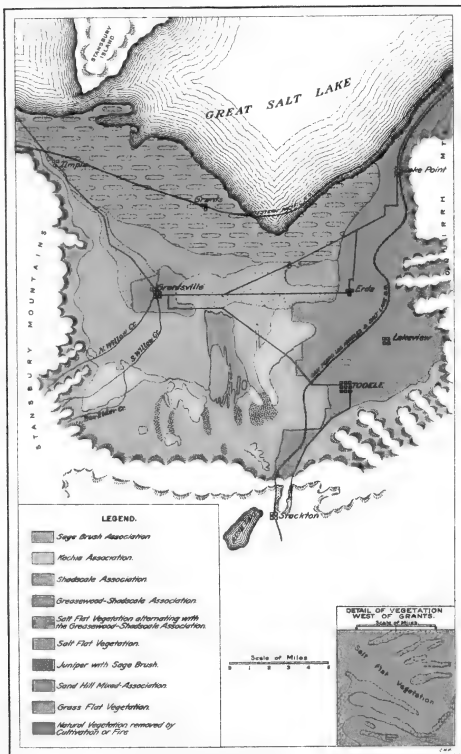
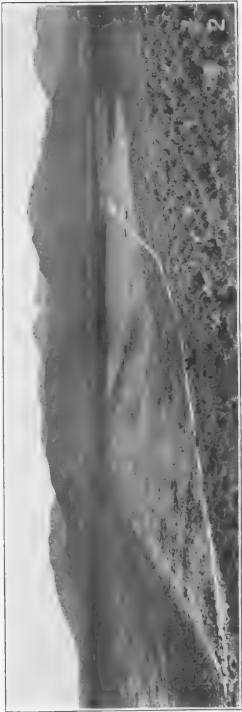
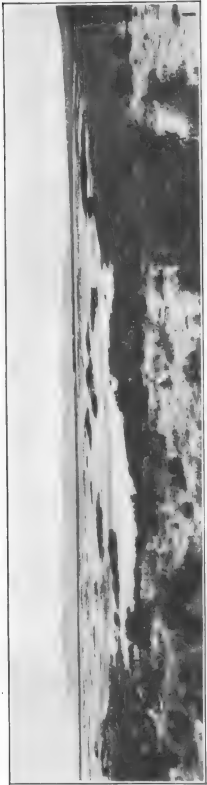


PLATE XLIII. Fig. 1.—Salt-flat vegetation bordering Great Salt Lake with a grease-wood-shadscale ridge in the foreground, a pure stand of *Salicornia utahensis* at the right and hummocks covered with *Allenrolfea occidentalis* in the background.

Fig. 2.—Sagebrush association (the darker areas) and islands of *Kochia* vegetation (the lighter areas) in the upper part of Tooele Valley. The sagebrush is encroaching upon the *Kochia* (at left).



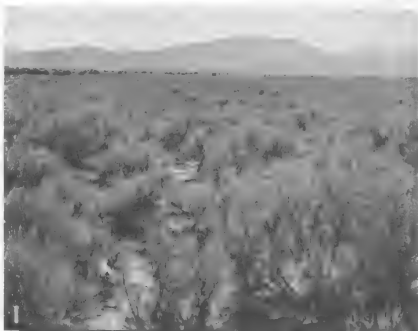


PLATE XLIV. Sagebrush (*Artemisia tridentata*). Fig. 1.—A good stand and growth, showing the typical appearance of this association where the conditions are relatively favorable. *Juniperus utahensis* in the background.

Fig. 2.—Plants showing the root habit; photographed at the edge of a deep "arroyo" where the soil had recently caved in. The extensive development of the lateral roots in the upper soil and the penetration of the taproot to a depth of about 11 feet is illustrated.

PLATE XLV. Fig. 1.—Sagebrush land which has recently been burned over, showing scattered, dead plants of *Artemisia tridentata* (no living ones), a dense growth of the annual grass *Bromus tectorum*, and scattered plants (dark colored in the picture) of *Gutierrezia sarothrae*.

Fig. 2.—An advanced stage in succession on sagebrush land which has been under cultivation, with numerous young plants of *Artemisia tridentata* and a dense herbaceous covering of *Bromus tectorum* and alfalfa (*Erodium cicutarium*).

Fig. 3.—Sagebrush reestablished on land which has been in cultivation (right) and the original, undisturbed sagebrush vegetation (left). The Stockton embankment in the background.





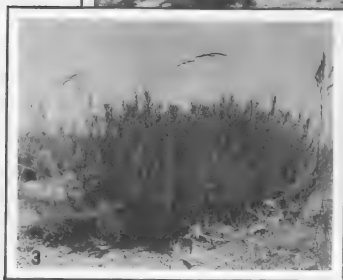


PLATE XLVI. Fig. 1.—Line of contact between the sagebrush association (right hand) and the *Kochia* association (left hand), showing the characteristically sharp demarcation of the two types. Soil samples collected at each side of this line, at points only 20 feet apart, showed that in the *Kochia* land there was ten times as much salt in the first foot and seventy-five times as much in the second foot as in the sagebrush land.

Fig. 2.—A typical view of the *Kochia* association, with plants rather far apart, and very uniform in size and appearance. This land has been pastured, which has resulted in the removal of practically all grasses and other species which occur in this association when protected from grazing animals.

Fig. 3.—Plants of *Kochia vestita*, 4 or 5 inches high, and the grass *Poa sandbergii*, which is usually associated with the *Kochia* in land that is not grazed.

- PLATE XLVII. Fig. 1.—Typical shadscale vegetation, consisting of a nearly pure stand of *Atriplex confertifolia*, showing much dead wood, as is usually the case, but the stand is denser than in much of the area occupied by this association.
- Fig. 2.—Transition area between the shadscale and the greasewood-shadscale types of vegetation. Scattered (larger and darker colored) plants of greasewood (*Sarcobatus vermiculatus*) in an area occupied chiefly by shadscale.
- Fig. 3.—Salt grass (*Distichlis spicata*) covering the whole of the depression to the right with the exception of a colony of *Allenrolfea* in the middle distance. The higher land to the left is occupied by greasewood (very dark in the illustration) and shadscale.





- PLATE XLVIII. Fig. 1.—Salt-flat vegetation, *Allenrolfea* community. The ground between the hummocks is covered with a white crust of salts, mostly sodium chlorid.
- Fig. 2.—Salt-flat vegetation, showing plants of *Salicornia utahensis*.
- Fig. 3.—Grass-flat vegetation, *Sporobolus-Chrysothamnus* community, showing a species of rabbit brush, associated with tussock grass.





# CITROPSIS, A NEW TROPICAL AFRICAN GENUS ALLIED TO CITRUS

By WALTER T. SWINGLE, *Physiologist in Charge*, and MAUDE KELLERMAN, *Botanical Assistant, Crop Physiology and Breeding Investigations, Bureau of Plant Industry*

## INTRODUCTION

Missionaries and pioneer explorers of equatorial Africa long ago reported the finding of wild oranges and wild lemons. If the fruits were green, they resembled small limes and lemons; if ripe, their sweet and agreeable flavor caused them to be classed as oranges.

These fruits are from 2 to 3 cm. in diameter and are borne, two to five or more in a cluster, in the axils of the leaves. Because of this pecul-



FIG. 1.—*Citropsis Schweinfurthii*: A branch showing 3-foliate and 5-foliate leaves, leaflike petioles, and rachis segments; also paired and single spines in the axils of the leaves. From a plant in greenhouse of the Department of Agriculture grown from seed from Budongo Forest, Uganda, Africa. (C. P. B. No. 2902.) One-fourth natural size.

iarly they may be called African cherry oranges. The leaves are odd-pinnate, usually with five leaflets, but often trifoliate. The petioles and the segments of the rachis are so broadly winged that in some species they look not unlike leaflets. (See fig. 1.)

As early as 1870 Schweinfurth, the veteran African explorer, had collected leafy twigs of one of these plants, but no flowers or fruits, in the

"Galleriewaldungen" at Uando, near the divide between the Congo and the Bahr-el Ghazal drainage basins. In 1880 Soyaux collected specimens of another species in Gabun (French Congo). In 1882 Pogge collected material at Lulua in Congo proper, and in 1890 Preuss found still another very distinct species on the shores of Elephant Lake in Kamerun. Early in 1895 Prof. Adolph Engler described four new species of *Limonia* to include these plants.<sup>1</sup> In November of the same year he segregated these African species of *Limonia* as a new section, *Citropsis*, in contradistinction to the true *Limonias* of the Asiatic mainland.<sup>2</sup>

Since then several additional species have been described from tropical Africa, and it is now clear that these plants occur not uncommonly throughout central Africa from the Ivory Coast in the west to Uganda in the east.

In connection with a study of the plants related to *Citrus*, these African species of the *Citropsis* section of *Limonia* have been carefully examined. The material of this section in the principal European collections of African plants has been studied and a number of representative specimens secured, through the generosity of M. Émile de Wildeman, of Brussels, and M. Auguste Chevalier, of Paris. Mr. B. T. Dawe, formerly Forest Administrator of Uganda, who had discovered a new species (*Limonia ugandensis* Baker) in the forests bordering the north shore of Victoria Nyanza, sent to the Department of Agriculture at Washington in 1910 both good herbarium specimens and viable seed.

As a result of these investigations, which have been in progress some three years, it is now clear that these plants have been wrongly placed in the Asiatic genus *Limonia*. Instead of constituting a section of this genus, they are in reality only remotely related to the type species from Asia (*Limonia acidissima* L.) and are, on the other hand, closely and clearly related to *Citrus*.

The *Limonia acidissima* (*Hesperethusa crenulata* (Roxb.) Roem.) of India has small, globose fruits only 12 mm. or less in diameter, becoming a purple-black, bitterish berry when ripe. Each of the four cells of the fruit contains a single seed surrounded with mucilage. There are no pulp vesicles. The fruits are, thus, of an entirely different structure from *Citropsis* and are like those of many Asiatic genera, such as *Lavanga*, *Triphasia*, *Severinia*, etc., which constitute a natural group.

Besides the very important differences in the structure of the fruit, *Limonia acidissima* differs from *Citropsis* in having free-spreading stamens with slender filaments. None of the other Asiatic species usually referred to *Limonia* are any more closely related to *Citropsis* than is *Limonia acidissima*.

<sup>1</sup> Engler, A. Diagnosen neuer Arten. In Notizbl. K. Bot. Gartens u. Mus. Berlin. Bd. 1, No. 1, p. 28-29. Jan. 2, 1895.

<sup>2</sup> Engler, A. Rutaceæ. In Engler, Adolf, and Prantl. Natürlichen Pflanzenfamilien. T. 3, Abt. 4, p. 189-190, fig. 109, E-H. Leipzig, 1895.

That the African species of *Limonia* constituting the section *Citropsis* are related to *Citrus* rather than to the Asiatic species of *Limonia* is a conclusion, based at first on a study of herbarium and living material, that has since been confirmed in gratifying manner by the results of experiments in grafting, which show that the African species belonging to the section *Citropsis* can be budded easily and grow well on all the commonly cultivated species of *Citrus*.

#### TECHNICAL DESCRIPTION OF CITROPSIS

It seems necessary to establish a new genus to include these African cherry oranges. This is best done by raising to generic rank the section *Citropsis* of Engler.<sup>1</sup>

**Citropsis** (Engler) Swing. and M. K.

The genus *Citropsis* resembles *Citrus* in the general structure and appearance of the flowers and fruit, as well as in the texture, venation, and general type of the leaves. It differs from *Citrus* in having 4- or rarely 5- merous ovaries, with only a single ovule in each cell; fruits with sessile pulp vesicles which are broad at the bases where they are embedded in the endocarp; the stamens only twice as numerous as the petals; large compound leaves; and spines usually occurring in pairs. The leaves are odd pinnate, 5- or rarely 7-foliate, trifoliate, or sometimes unifoliate, subcoriaceous, pellucid punctate. The spines are paired or single in the axils of the leaves. The flowers occur in few- or many-flowered axillary clusters and are perfect, 4- or rarely 5- merous. The stamens are twice as numerous as the petals, free but flattened, and arranged to form a staminal tube surrounding the pistil much as in *Citrus*. The disk subtends and is slightly larger than the base of the ovary. The ovaries are 4- rarely 5-celled with one ovule in each cell. The style is long and deciduous; the stigma is large, subglobose, more or less 4- rarely 5-lobed. The fruit is globular or subglobular, small (2 to 3 cm. in diameter), with a fleshy skin like that of a lime, dotted with oil glands. The pulp is vesicular, either sweet and edible or waxy. The pulp vesicles are not stalked as in *Citrus*, but are broad at the base where they are embedded in the endocarpic lining of the cells and taper gradually toward the pointed tips. In some species they are full of juice, in some they contain a waxy substance, and in some they dry up as the fruit develops. The seeds are large, 10 by 6 by 4 mm., with a hard, parchmentlike testa having a foramen at the tip. The cotyledons remain hypogeous in germination. The first two foliage leaves are opposite, as in *Citrus*.<sup>2</sup> (See fig. 2.)

<sup>1</sup> *Citropsis*, gen. nov. (*Limonia*, § *Citropsis*, Engler).—Genus *Citro* affinis, foliis pinnatis, staminibus paucioribus (staminum numero petalorum duplo nunquam quadruplo), ovariis 4- rarius 5- locularis, loculis monospermis.

Folia imparipinnata, trifoliata vel rarius unifoliata, subcoriacea, pellucido-punctata. Spinae in axillis foliorum geminae vel singulae. Paniculae axillares, pauciflores. Flores hermaphroditi, 4- vel rarius 5- meri. Stamina 8 vel 10 (numero petalorum duplo). Discus ovarii basin subtendens. Ovarium 4- vel rarius 5- locularis, stylus longus, deciduus, stigma plus minusve quadrilobum, ovulo in loculo singulo. Fructus globosus vel subglobosus, cortice ut in *Citro* carnosus, glandulis oleiferis instructo, pulpa vesiculari, dulci et eduli, vel cerea, vesiculis fusiformibus, ad basin in endocarpio immersis. Semina magna, testa dura, pergamena, foraminea. Cotyledones in germinatione hypogaeae.

Arbor parva vel arbuscula, spinosa.

Species typica, *Limonia Preussii* Engler.

<sup>2</sup> In *Citropsis Schweinfurthii* the first two postcotyledonary leaves are opposite, broadly oval, and short stalked; the next two or three leaves are simple, with short petioles; then follow unifoliate leaves with winged, longer petioles; then trifoliate leaves; and finally pinnately 5-foliate leaves. (See fig. 2.)

Shrubs or small trees; native to tropical Africa.

The type species is *Limonia Preussii* Engler, from Kamerun.

Citropsis is related to Citrus on the one hand and to Atalantia on the other. It differs from both in its compound leaves and broad-based pulp vesicles partly embedded in the endocarp and from Atalantia in

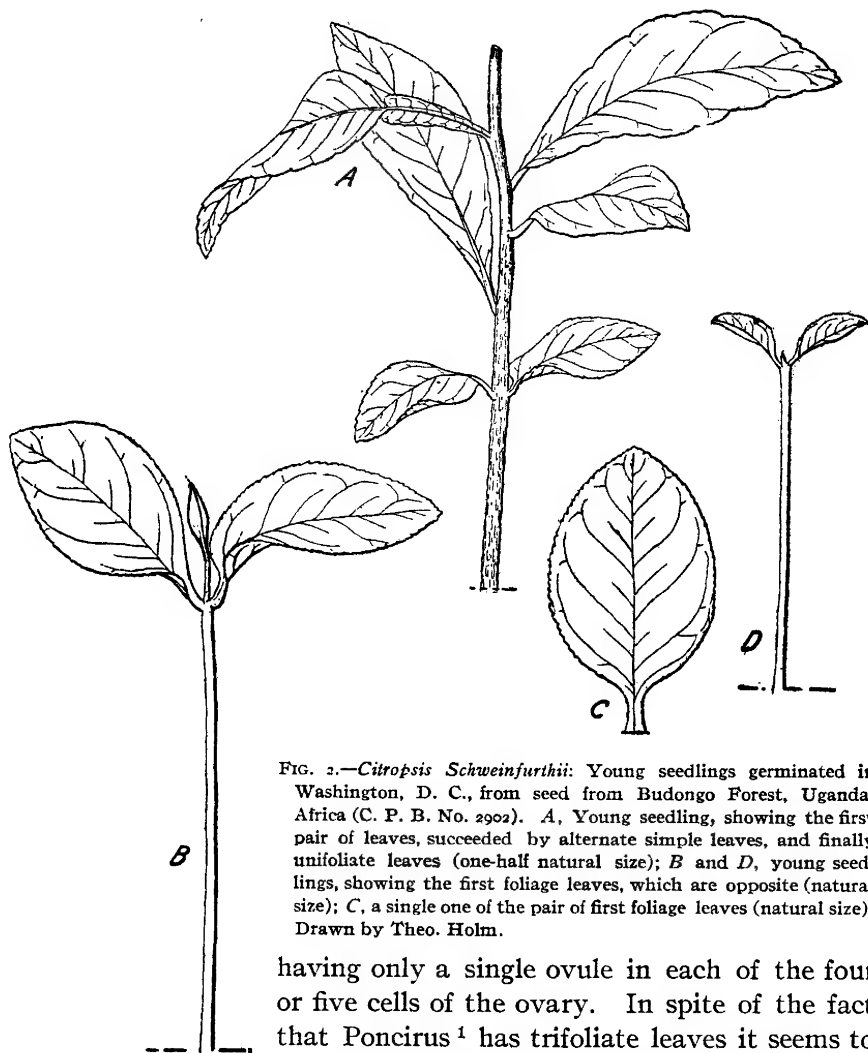


FIG. 2.—*Citropsis Schweinfurthii*: Young seedlings germinated in Washington, D. C., from seed from Budongo Forest, Uganda, Africa (C. P. B. No. 2902). A, Young seedling, showing the first pair of leaves, succeeded by alternate simple leaves, and finally unifoliate leaves (one-half natural size); B and D, young seedlings, showing the first foliage leaves, which are opposite (natural size); C, a single one of the pair of first foliage leaves (natural size). Drawn by Theo. Holm.

having only a single ovule in each of the four or five cells of the ovary. In spite of the fact that *Poncirus*<sup>1</sup> has trifoliate leaves it seems to be less closely related to *Citropsis* than is *Citrus*.

*Poncirus* differs from both *Citrus* and *Citropsis* in its deciduous leaves, sessile solitary flowers, clawed petals, spreading stamens, stalked pulp vesicles with external, branched, secreting hairs, and in having in germination the first postcotyledonary leaves in the form of alternate cataphylls.

<sup>1</sup> *Poncirus* Raf. includes *Citrus trifoliata* L., the type species, and as yet the only one known. See Swingle, Walter T. *Poncirus* (and *Citrus*). In Sargent, C. S. *Plantæ Wilsonianæ*. pt. 5. Cambridge, 1914.

## CHARACTERS WHICH DISTINGUISH SPECIES OF CITROPSIS

The principal diagnostic characters of the species of *Citropsis* are found in the flowers, leaves, and fruits. The size, shape, and proportions of the pistil and in particular of the style are of great importance. The smoothness or hairiness of the filaments and the shape of the ovary are also important characters, as is the length of the pedicel and peduncle in relation to the length of the pistil. The shape, size, and proportions of the leaflets, segments of the rachis, and petioles are not only obvious but necessary characters for use in distinguishing the species. Finally, the nature of the fruit, whether dry or pulpy, and if pulpy, whether juicy and sweet, or waxy, is useful in distinguishing the species. Owing to the number of species of *Citropsis* and the variability due to their wide range, it is usually necessary to have at least good flowers and leaves to be able to determine the species with any certitude, and in some cases fruits also are necessary.

Inasmuch as none of the original descriptions of the African species of *Limonia* now referred to *Citropsis* included both flowers and mature fruits, it is obvious that it is a matter of much difficulty to determine the affinities of some of these species based on imperfect material.

***Citropsis Preussii* (Engler), n. comb.**

*Limonia Preussii* Engler, 1895, in Notizbl. K. Bot. Gartens u. Mus. Berlin, Bd. 1, p. 28.

Illus., Engler, 1895, in Engl. and Prantl, Pflanzenfam., T. 3, Abt. 4, p. 189, fig. 109, E-H.

The following specimens<sup>1</sup> have been consulted: **Kamerun.**—PREUSS (No. 548), September 19, 1890, Barombi Station on Elephanten See (Dahlem Herbarium<sup>2</sup>; Kew Herbarium). STANDT (No. 747), November 29, 1896, Johann Albrechtshöhe (Dahlem Herbarium, fragment in National Herbarium, Washington, D. C.; British Museum Herbarium). BÜSGEN (No. 37), November 18, 1905, Johann Albrechtshöhe (Dahlem Herbarium). LEDERMANN (No. 1455), December 1, 1908, Bare (Dahlem Herbarium; fragment in National Herbarium, Washington, D. C.).<sup>3</sup>

The type of the genus, *Citropsis Preussii*, was first collected by Preuss at Barombi Station on the south shore of Elephanten See in Kamerun on September 19, 1890. Of his original collection (No. 548) three specimens, all showing good flowers, have been studied by the writers. (See fig. 3.) Two of these are preserved in the herbarium at Dahlem, near Berlin. The third was sent to Kew Gardens (April, 1894) before the species was published and evidently was not used by Prof. Engler in drawing up the original description, as the species is described as having trifoliate leaves, while those of the Kew specimen are 5-foliate.

Besides this original material there are three excellent sheets in the Dahlem Herbarium and one at South Kensington of material collected by Standt (No. 747) on November 29, 1896, at Johann Albrechtshöhe,

<sup>1</sup> All of the specimens cited from European herbaria were photographed by one of the writers in 1911-12, and prints enlarged to natural size have been filed in the National Herbarium at Washington, D. C.

<sup>2</sup> The sheet to which the original label is attached is the type specimen.

<sup>3</sup> Ledermann's specimens have been designated "*Limonia Preussii* Engl., var. *micrantha* Engl.," but it is probable that the very small flowers are due to a diseased condition of the plant and do not constitute a true varietal difference.

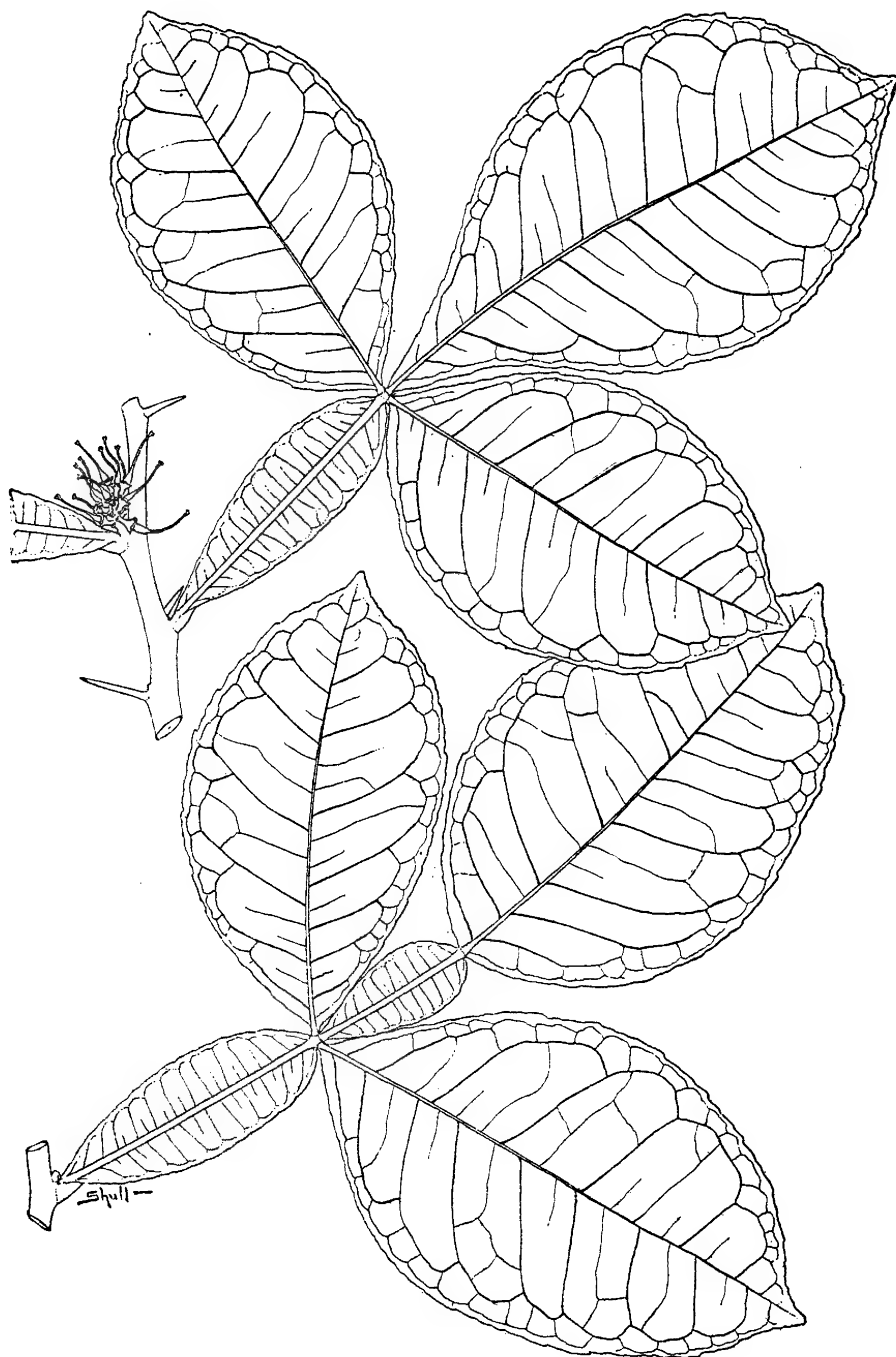


FIG. 3.—*Citropsis Preussii*: Flowers after petals and stamens have fallen; leaves, one trifoliate and one having the terminal leaflet borne on a winged segment of the rachis. From paratype, Standt No. 548, in Dahlem Herbarium. One-half natural size.

near the original type locality on Elephanten See. These specimens show flowers and young fruits. Finally, there is one sheet in the Dahlem Herbarium, collected by Büsgen (No. 37) on November 18, 1905, also at Johann Albrechtshöhe near Elephanten See. This specimen shows young fruits.

All of this material comes from the same general locality, Johann Albrechtshöhe being only 3 or 4 km. distant from Barombi Station. All eight of these specimens show a great resemblance and undoubtedly belong to a single species. Unfortunately all were collected in the autumn and show only flowers and very young fruits.

A number of other specimens have been referred to *Citropsis Preussii* in the Dahlem Herbarium, but some of them certainly do not belong here, and for the present the only material certainly referable to this species is that collected in the immediate vicinity of Elephanten See in Kamerun.

The excellent specimens with flowers and young fruit and numerous leaves permit a very good idea to be gained of this species.

The leaves are 3- to 5-foliate, with broadly winged petiole and rachis. (See fig. 3.) The leaflets are very large, 100 to 160 by 45 to 115 mm., broadly oval or oblong, abruptly narrowed above into a short obtuse tip, and broadly cuneate at the base, with very short petiolules. Petioles usually 69 to 80 by 25 to 35 mm., elongate, elliptical, rather acute at tip and base, but sometimes shorter and broader or even obcordate 30 to 40 by 25 mm. The segments of the rachis are elongate elliptical, 50 to 70 by 15 to 25 mm. Spines usually single, 16 to 28 mm. long, rarely wanting. Flowers 15 to 18 mm. long in the bud, 20 to 25 mm. in diameter when open, in dense many-flowered clusters borne in the axils of the leaves, very short pediceled (3 to 5 mm.), usually 4-merous, ovaries 12 to 15 mm. long, with a long, slender style broadening at the base and merging gradually into the ovary. Only young fruits are known as yet. These are short-stalked or nearly sessile, slightly apiculate.

*Citropsis Preussii* is readily distinguished from its congeners by the broadly oval or oblong leaflets, and by the short-stalked flowers with very long styles broadened at the base and not sharply delimited from the tip of the pointed ovaries. *Citropsis mirabilis* resembles this species in the shape of the leaves, winged petioles, and rachis, but differs in the longer stalked flowers, which have a shorter more slender style which is not broadened at the base and consequently is more sharply delimited from the tip of the more rounded ovary. (See fig. 4.)

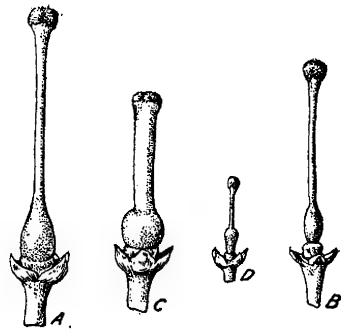


FIG. 4.—Pistils of four species of *Citropsis*. A, *Citropsis Preussii* (Standt No. 747); B, *Citropsis mirabilis* (Chevalier No. 21609); C, *Citropsis Schweinfurthii* (C. P. B. 2902); and D, *Citropsis gabonensis* (Klaine No. 2260). Twice natural size.

**Citropsis Schweinfurthii** (Engler), n. comb.

*Limonia Schweinfurthii* Engler, 1895, in Notizbl. K. Bot. Gartens u. Mus. Berlin, Bd. 1, p. 29.

(?) *Limonia ugandensis* Baker, 1907, in Jour. Bot. [London], v. 45, p. 61.

(?) *Limonia Poggei* Engler, 1895, in Notizbl. K. Bot. Gartens u. Mus. Berlin, Bd. 1, p. 29.

The following specimens have been consulted: **Sudan**.—SCHWEINFURTH (No. 3656) April 25, 1870, Uando (Dahlem Herbarium,<sup>1</sup> clastotype in National Herbarium, Washington, D. C., see fig. 7; Kew Herbarium, clastotype). STUHLMANN (No. 2641), August 24, 1891, Ituri Ferry (Dahlem Herbarium). **Uganda**.—BAGSHAW (No. 1007),<sup>2</sup> April 25, 1906, Mpanga Forest, Toro (British Museum Herbarium); (No. 1365), December 17, 1906, Ngusi River, Albert Edward Nyanza, altitude 950 meters (British Museum Herbarium; National Herbarium, Washington, D. C.). DAW (No. 399), South Buddu (Kew Herbarium); (No. 809), 1905, Budongo Forest (Kew Herbarium); (No. ?) March 17, 1910, Budongo Forest (National Herbarium, Washington, D. C.); (No. ?, C. P. B. No. 2902) April 17, 1910, Budongo Forest (National Herbarium; greenhouses, Department of Agriculture, Washington, D. C. See fig. 1 and Pl. XLIX). MILBRAED (No. 2394) January 1, 1908, Fort Beni (Dahlem Herbarium); (No. 2880), May 1, 1908, Irumu (Dahlem Herbarium). **Congo**.—POGGE (No. 668),<sup>3</sup> June 1, 1882, Lulua (Dahlem Herbarium). LAURENT (No. ?), November 24, 1903, Ibaka (Brussels Herbarium); (No. ?), January 2, 1904, Bolombo (Brussels Herbarium; National Herbarium, Washington, D. C.); (?) **French Congo**.—THOLLON (No. 1049), June, 1888, on Niari River from Komba to Bounanza (Muséum, Paris, Herbarium).

In 1895 Engler published *Citropsis Schweinfurthii*, which was based on a single unbranched twig without flowers or fruit collected by Schweinfurth (No. 3656) in April, 1870, in the "Galleriewaldungen" at Uando (altitude 700 to 800 m.; lat. 4° 18' N., long. 28° 22' E.), about 260 km. northeast of Albert Nyanza. The twig was originally some 33 cm. long, with 12 internodes. The basal internode, with a trifoliate leaf, was sent to Kew Herbarium in February, 1878, where it is now preserved. The rest of the specimen is in Prof. Schweinfurth's herbarium in the Dahlem Museum and is the type upon which Prof. Engler based the species. In the original description of the species the leaves are said to be trifoliate, but in this specimen one of them, the fifth from the tip of the twig, is pinnately 5-foliate with a well-developed, broadly winged rachis between the first and second pair of leaflets. One of the lateral leaflets of the terminal pair is missing, but the shape and position of the terminal leaflet show clearly that it was present during the life of the plant and was probably lost after the specimen was dried, as has happened to seven or eight leaflets belonging to other leaves of this same specimen.

The discovery of this pinnate leaf on the type specimen is of importance in justifying the reference to this species of a number of pinnate-leaved specimens from the eastern part of equatorial Africa.

A fruiting specimen was collected at a ferry of the Ituri River about 60 km. WNW. of Albert Nyanza in latitude 2° 55' N. (altitude 900 meters) by Dr. F. Stuhlmann (No. 2641) on August 24, 1891, in his journey around the great lakes of equatorial Africa. Stuhlmann mistook the

<sup>1</sup> This is the type specimen.

<sup>2</sup> Type specimen of *Limonia ugandensis*.

<sup>3</sup> This is the type specimen of *Limonia Poggei*.



broadly winged segments of the rachis for leaflets sprouted out of each other.<sup>1</sup> His specimen is preserved in the Dahlem Herbarium and has been referred to *Limonia Schweinfurthii* by Engler.<sup>2</sup>

The original label has a note by Stuhlmann to the effect that the fruit is orangelike, light yellow in color, shows two seeds, and has a sweet pulp without acid. A sketch on the label shows a 4-celled fruit with two seeds. Most of the leaves are pinnately 5-foliate, though the specimen is in bad condition and many leaflets have been lost. Both the leaves and spines are much like those of Schweinfurth's original specimen from Uando, and it is very probable that both belong to the same species.

Misled by the statement in the original description of this species that the leaves are trifoliate, Baker described a new species, *Limonia ugandensis*, in 1907, which he says differs from *Limonia Schweinfurthii* (known to him only from the description) in having 5-foliate instead of 3-foliate leaves.

The type of *Limonia ugandensis* was collected by Mr. A. G. Bagshawe (No. 1007) on April 25, 1906, at Toro, in the Mpanga Forest, to the east of Albert Nyanza, in western Uganda, at an altitude of 1,550 meters. The type specimen shows flower buds and has single spines and mostly 5-foliate leaves, but apparently a few 3-foliate leaves also. The petioles and segments of the rachis are broadly winged and vary from narrowly elliptical to obovate in outline. Because of the absence of mature flowers the description of the stamens is erroneous in giving the filament as about equaling the anther in length. In a fully open flower the filaments would undoubtedly be much longer. A specimen of this species which was collected by Mr. A. G. Bagshawe (No. 1365) at Ngusi River, Albert Edward Nyanza, at an altitude of 970 meters, shows good fruits (see fig. 5).

Aside from the usually but not universally broader winged petiole and rachis segments these specimens can scarcely be distinguished from *Citropsis Schweinfurthii*, and unless the flower and fruit characters prove to be different, *Limonia ugandensis* will doubtless have to be considered to be a synonym of *C. Schweinfurthii*.

Besides the specimens from Uganda hitherto referred to *Limonia ugandensis* Baker, there are two specimens in the Dahlem Herbarium,

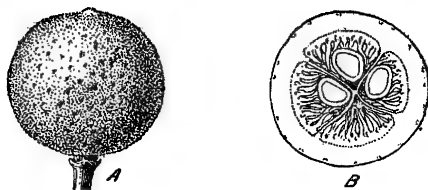


FIG. 5.—*Citropsis Schweinfurthii*: Nearly mature fruit; A, side view, showing calyx and disk; B, section showing four cells with pulp vesicles and three seeds. Bagshawe No. 1365, in National Herbarium, Washington, D. C. Natural size.

<sup>1</sup> "In dem dichten Unterholz fiel uns vor allem ein kleiner Busch mit dornigen Aesten auf. Von seinen lederharten Blättern spriesst eines aus dem anderen heraus. Seine Frucht ist eine kleine Orange mit mehreren Abtheilungen, aber nur zwei Kernen. Von unseren Limonen unterscheiden sie sich durch den süßlichen, jeder Säure entbehrenden Geschmack." (Stuhlmann, Franz. Mit Emin Pascha ins Herz von Afrika. p. 406. Berlin, 1894.)

<sup>2</sup> Engler, Adolf. Die Pflanzenwelt Ost-Afrikas. . . . Teil C, p. 229. Berlin, 1895.

collected by J. Milbraed in 1908, which seem to be referable to *Citropsis Schweinfurthii*. One specimen (No. 2394) is from Fort Beni, in extreme western Uganda, on the Semliki River, about half way between Albert Nyanza and Albert Edward Nyanza. This specimen consists of a single twig with 5-foliate leaves, single spines, and two young fruits. The other (No. 2280) is from Kikufu, near Irumu, in the Ituri River valley, only a few kilometers south of the ferry where Stuhlmann crossed the Ituri and collected his No. 2641. This second specimen of Milbraed consists of two twigs with mostly 5-foliate leaves, but one of them has a trifoliate leaf almost exactly like those of Schweinfurth's original specimen from Uando.

*Limonia Poggei* Engler, which the writers have referred doubtfully to *Citropsis Schweinfurthii*, was based on a single specimen collected by Pogge (No. 668) June 1, 1882, at Lulua, latitude 6° S., on the Lulua River, an affluent of the Kasai River. The type specimen preserved in the Dahlem Herbarium shows a single twig with 11 or 12 internodes, but with only one 5-foliate leaf remaining attached. There is also one loose leaf and a single fruit. Pogge's original label notes that the fruit is yellow. An examination of the fruit preserved with the type specimen at Dahlem shows it to possess distinct pulp vesicles. There is nothing in the specimen or in the description to distinguish it from *Citropsis Schweinfurthii*, and as it occurs at a considerable altitude, 660 meters, and only 500 km. west from the nearest of the great African lakes, while Uando, the type locality, was some 250 km. west, its geographic range is not such as to render its inclusion in the species improbable.

It is interesting to note that all the reported localities of *Citropsis Schweinfurthii* are above 660 meters altitude, the highest reported being 1,550 meters at Toro, Mpanga Forest, Uganda.

There is, however, a specimen in the herbarium of the Muséum d'Histoire Naturelle at Paris, collected by Thollon (No. 1049) in June, 1888, in French Congo on the Niari River between Bounanza and Komba, that can scarcely be distinguished by its leaf characters from *Citropsis Schweinfurthii*. Bounanza is only 250 km. from the Atlantic Ocean and at an altitude of only 130 meters. Thollon states on his original label that this plant occurs in all the woods from Komba to Bounanza. If this material proves to be *Citropsis Schweinfurthii*, it will give this species the greatest range both in distance and in altitude of any yet known in the genus *Citropsis*.

There is a specimen<sup>1</sup> in the herbarium of the botanic garden at Brussels, collected by Messrs. Ém. and M. Laurent below Ibaka, on the Sankuru River, Congo, on November 24, 1903, and also a specimen in the National Herbarium at Washington, D. C., collected by Messrs. Laurent below Bolombo, on the Sankuru River, on January 2, 1904.

<sup>1</sup> This specimen seems to have been referred to *Limonia Demeusei* by M. Émile de Wildeman. See his *Mission Émil Laurent* (1903-4). v. 1, p. 238. Brussels, 1905-1907.

Both of these specimens, as well as one in the herbarium at Brussels, collected in Congo by Messrs. Laurent in 1903-4, but without exact locality or date, have trifoliate leaves, long, slender leaflets, with the terminal one disproportionately long, being 110 to 165 by 30 to 45, while the adjacent lateral leaflets are 65 to 90 by 25 to 45; thus the terminal leaflet is from two-fifths to one-third longer than the lateral. The only species to which these specimens can be referred at present is *Citropsis Schweinfurthii*, but in the absence of flowers and fruits and because of the rather unusual appearance of the leaves such reference must be merely provisional.

*Citropsis Schweinfurthii* is a spiny shrub or small tree with 3- to 5-foliate leaves. The flowers are borne in clusters of 4 to 10 in the axils of the leaves. (See fig. 6.)

They are 4- or rarely 5-parted with strap-shaped petals, a short, thick style, 6 to 9 mm. long, scarcely narrower than the stigma but rather sharply set off from the rounded tip of the ovary, and broad flattened filaments with a subulate apex where the anther is attached. The leaves are pinnately 5-foliate or trifoliate. The petioles are broadly winged, 40 to 75 by 18 to 35 mm., narrowly obovate or elliptical, usually rounded at the tip and bluntly pointed at the base. The segments of the



FIG. 6.—*Citropsis Schweinfurthii*: Cluster of flowers, showing stamens arranged to form a staminal tube. From a plant growing in greenhouse of the Department of Agriculture, grown from seed from Budongo Forest, Uganda, Africa. (C. P. B. No. 2902.) Natural size.

rachis are 35 to 65 by 15 to 25 mm., usually elliptical, bluntly pointed at both ends but more rounded (sometimes rather broadly rounded) at the tip. The leaflets, 55 to 125 by 15 to 50 mm., are broadly lanceolate, narrowed from the middle to the long, cuneate base and into an acute or subacute tip, with strongly marked serrations. (See fig. 7.) The terminal leaflet is often much larger than the adjacent lateral leaflets, sometimes one-third longer, usually from one-fourth to one-eighth longer. The spines, 12 to 30 mm. long, are usually paired in the axils of the leaves.

*Citropsis Schweinfurthii* differs from all its congeners in having a short, thick style (shorter than any other species except *C. gabunensis*, which has very small flowers, with a slender style) and slender, broadly lanceolate leaflets, narrowing from the middle into a long, cuneate acute base.

***Citropsis gabunensis* (Engler), n. comb.**

*Limonia gabunensis* Engler, 1895, in Notizbl. K. Bot. Gartens u. Mus. Berlin, Bd. 1, p. 28.

(?) *Limonia Lacourtiana* De Wild., 1904, in Ann. Mus. du Congo, Bot. s. 5, v. 1, p. 159-160, pl. 50. Illus., De Wild., op. cit., pl. 50.

The following specimens have been consulted: **French Congo** (Gabun).—SOYAUX (No. 105), July 25, 1880, Sibanga Farm, Munda. (Dahlem Herbarium,<sup>1</sup> Kew Herbarium; Muséum, Paris, Herbarium). KLAINE (No. 2260), July and October, 1901,



FIG. 7.—*Citropsis Schweinfurthii*: A trifoliate leaf from the type specimen, showing double spines in the axils and pronounced serrations of the leaflets toward the tips (Schweinfurth No. 3656); in National Herbarium, Washington, D. C. Natural size.

near Libreville (Kew Herbarium; Dahlem Herbarium; Muséum, Paris, Herbarium); (No. 1973), March 10, 1901, May and October, 1902, Libreville (Muséum, Paris, Herbarium); (No. 2924, 2925), June, 1902, Libreville (Muséum, Paris, Herbarium); (No. 3494), May 25, 1904, Libreville (Muséum, Paris, Herbarium). BUTNER (No. 432),

<sup>1</sup> The specimen with the original label attached is the type.

September, 1884, Sibange Farm (Dahlem Herbarium). DU BELLAY (No. 4?), 1864 (Muséum, Paris, Herbarium). TESSMANN (No. 874), January 26, 1909. **Spanish Guinea** (P)—Bebady (?); (No. 194), February 14, 1908, Nkolentagan. (P) **Congo**.—GENTIL (No. 93),<sup>1</sup> May, 1893, Bombaie (Brussels Herbarium). HENDRICKX (coll. Gillet, No. 3280) Lumene (Brussels Herbarium). LAURENT (No. ?), November 28, 1903, Bombaie (Brussels Herbarium).

*Citropsis gabunensis*, one of the first four species of *Limonia*, described from Africa by Engler in 1895, was based on specimens collected by H. Soyaux (No. 105) at Sibanga Farm in the Munda region near Libreville, French Congo (Gabun), on July 25, 1880. Three sheets of this number are preserved in the Dahlem Herbarium, and on them the species is based. The type specimen had a single fruit; the paratypes are sterile. The herbaria of Dahlem, Brussels, Paris, and Kew contain numerous other specimens of this species from northern French Congo and Spanish Guinea. This material represents a wide range of foliar characters and shows all stages of flower and fruit development. All these specimens seem to belong to a single species which is very distinct from any of the others.

The type specimen of *Limonia Lacourtiana* was collected by L. Gentil (No. 93), May, 1893, and is preserved along with Gentil's original label in the herbarium of the botanic garden at Brussels. The leaves are all 5-foliate, and in one case a terminal leaflet has a winged petiole. The leaflets are broadly oval, more or less abruptly narrowed at the base, and caudate at the tip. The young fruits are borne in clusters in the axils of the leaves on pedicels 10 to 12 mm. long. In all of these characters this specimen is indistinguishable from *Citropsis gabunensis*.

A young fruit from this type specimen now preserved in the National Herbarium at Washington, D. C., seems to be seedless, but shows numerous pulp vesicles which contain a whitish granular wax.<sup>2</sup> The original label of M. Gentil says "fruits délicieux," but as the fruits in the type specimen are very small and immature it is obvious that his statement must apply to some other plant, doubtless not belonging to this species. Most of the fruits of the typical *Citropsis gabunensis* examined contain large seeds, often nearly filling the small fruit and leaving very little space for the pulp vesicles, which are crowded and often nearly obliterated by the seeds.

Whether the vesicles of a young seedless fruit of the typical *Citropsis gabunensis* would show the presence of wax remains to be investigated. In the absence of knowledge on this point it seems inadvisable to recognize *Limonia Lacourtiana* as a species distinct from *Citropsis gabunensis*, though future research may possibly prove it to be a good species.

<sup>1</sup> This is the type specimen of *Limonia Lacourtiana*.

<sup>2</sup> Recently, through the kindness of M. Auguste Chevalier and of Rev. J. Gillet, of Kisantu, Congo, abundant material has been received of a species of *Citropsis* apparently distinct from any hitherto described, the fruits of which are often seedless and contain abundant pulp vesicles filled with a wax, which, when extracted, makes a yellow, fragrant mass much like beeswax in character.

*Citropsis gabunensis* differs from all its congeners in having very small flowers, with hairy filaments, caudate leaflets, and a nearly dry fruit. The flower buds are only 5 to 6 mm. long and the fully expanded flowers are only 10 to 12 mm. in diameter. The filaments are hairy. The pistil is very short ( $3\frac{1}{2}$  to 4 mm.) and shows a well-marked, clavate ovary, narrowed gradually toward the base and rounded at the tip, which is clearly delimited from the slender style which ends in the subglobose 4-lobed stigma. (See fig. 4.) The pedicels are very long (sometimes 8 to 12 mm.), often twice as long as the pistil, and appear as branches of a slender peduncle  $\frac{1}{2}$  to 2 cm. long.

No other species of *Citropsis* shows so much variation in the size of the leaves and in the number of leaflets. They may be unifoliate, greatly resembling orange leaves, or they may have 5 to 7 leaflets. Very frequently the leaves are 5-foliate, with the terminal leaflet borne at the end of a winged segment of the rachis. Such stalked terminal leaflets are often seen in trifoliate leaves (see fig. 3) but almost never in 5-foliate leaves of other species of *Citropsis*. The leaflets are caudate—unlike any of the other species.

Those of compound leaves are from 40 to 115 by 18 to 60 mm., mostly 50 to 100 by 25 to 45. The leaflets of unifoliate leaves are 90 to 150 by 40 to 70 mm. The winged petioles are 15 to 35 by 3 to 15 mm., varying from linear to narrowly obcordate, especially in unifoliate leaves. They are usually broadly rounded at the tip and narrowed gradually toward the base. The rachis segments vary from 20 to 45 by 4 to 10 mm. and usually have the same shape as the winged petioles.

***Citropsis mirabilis* (Chev.), n. comb.**

*Limonia mirabilis* Chevalier, 1912, in Bul. Soc. Bot. France, t. 58, 1911, Mém. 8d, p. 144-145.

The following material has been consulted: **Ivory Coast**.—CHEVALIER (No. 21609), May 21, 1909, between Sanrou and Quode on the Koue River (Chevalier Herbarium, Paris; National Herbarium, Washington, D. C.).

Chevalier has described *Citropsis mirabilis* in detail, but unfortunately no fruits are known. The leaves are 3- to 5-foliate, with broadly oval or oblong leaflets 90 to 190 by 40 to 100 mm. The petioles are usually elongate elliptical, 60 to 70 by 20 to 30 mm., rather acute at both ends, rarely broadly rounded at the tip. The segments of the rachis are 80 to 70 by 12 to 28 mm., usually narrowly elliptical, rarely broadly rounded at tip. The spines are single, 10 to 28 mm. long, sometimes wanting. The flowers occur in dense many-flowered clusters in the axils of the leaves. The pedicels are well-developed, 5 to 6 mm. long. The buds are linear elliptical, 12 to 14 by 3 mm., the flowers when open are 18 to 24 mm. in diameter, usually 4-merous, but sometimes 5-merous. The pistil is 12 to 14 mm. long, the style 10 to 11 mm. long, very slender, and not appreciably broadened at the base.

*Citropsis mirabilis* differs from all its congeners in having large flowers with slender styles not much broadened at the base and, in consequence, rather clearly delimited from the tip of the ovary. (See fig. 4.) It somewhat resembles *C. Preussii* in the size and shape of the leaves.

#### IMPERFECTLY KNOWN SPECIES

##### *Citropsis articulata* (Willd.), n. comb.

*Citrus articulata* Willd., 1826, in Spreng., Syst. Veg., v. 3, p. 334.

The following material has been consulted: **Gold Coast**.—ISERT (No. ?; Willdenow Herbarium No. 14357), June or July, 1786, near Kommang, Akwapim<sup>1</sup> (Dahlem Herbarium). (?) **Togo**.—BAUMANN (No. 552), 1894-5, on the Koli River near Kame (Dahlem Herbarium).

The specimen in the Willdenow Herbarium at Dahlem, of which a photograph was kindly sent to the writers by Prof. Urban, now shows a single twig, 21 cm. long and  $2\frac{1}{2}$  to  $4\frac{1}{2}$  mm. in diameter, with 10 or 11 internodes which are mostly 2 to  $2\frac{1}{2}$  cm. long, slightly angular, with prominent leaf scars. Only two single spines are preserved, one 8 by 1 mm., the other 14 by  $1\frac{1}{2}$  mm. Two petioles are on the sheet: One, still attached, obovate in outline, 52 by 32 mm. tapering gradually into the sharp base 4 to 5 mm. long; the other broadly rounded at tip, 60 by 37 mm. with prominent veins, running nearly at right angles to the midrib, the margin very shallowly undulate crenate. It is evident that the Isert specimen at Dahlem was more complete when Sprengel published Willdenow's description, as the leaves are said to be oblong and the peduncle many-flowered. Probably only a single terminal leaflet was originally present. The many-flowered peduncle seems also to have fallen off since Willdenow's time, as none can now be seen on the photograph.

To this species has been doubtfully referred a specimen from Togoland collected by E. Baumann (No. 552), on May 16, 1895, on the Koli River near Kame, probably not very remote from the locality where Isert's type was collected. The Baumann specimen has 3- to 5-foliate leaves, with petioles varying somewhat in size and shape, 35 by 13, 60 by 25, 32 by 50, 40 by 18, or 30 by 20 mm. Curiously enough, the terminal portion of the twig, including the last five or six internodes, has lost its leaves except one obovate petiole. It has three single spines and in general resembles in a striking manner Isert's specimen, upon which Willdenow based his species. Another curious coincidence is in the presence of the terminal leaflet of an originally trifoliate leaf from which the two lateral leaflets have fallen. It was probably from such an apparently unifoliate leaf originally present on Isert's specimen that

<sup>1</sup> Isert found this plant in the mountains some 50 to 75 km. north of Accra and says of it: "Je vis une nouvelle espèce de citroniers, avec des feuilles articulées." (Isert, P. E. Voyages en Guinée et dans les îles Caraïbes en Amérique, p. 255-256. Paris, 1793. A reprint of the original edition, Reise nach Guinea . . . 1788, appears in Allgemeine Geschichte der neuesten Reisen und Entdeckungen, v. 1.)

Willdenow described the leaves as oblong. This Baumann specimen shows an axillary inflorescence comprising some 6 to 8 flowers with slender ovaries (10 to 11 mm.) and very slender styles somewhat like *Citropsis mirabilis*. The leaves of the Baumann specimen have more broadly winged petioles than the *C. mirabilis*, and doubtless because of this it was referred in the Dahlem collection to *C. Preussii*, from which it differs in the distinctly shorter, more slender style, the narrow smaller leaflets, and the broadly rounded tips of the winged petioles and segments of the rachis. The flowers in the Baumann specimen are more densely clustered and shorter pediceled than in *C. mirabilis*.

It is to be hoped that more complete material collected by Isert may be found in the Copenhagen Herbarium which will permit the affinities of this species, the first of the group to be discovered, to be determined with exactitude.

Besides the foregoing, there remain two more African species of *Limonia* which undoubtedly belong to *Citropsis*, but which can not as yet be satisfactorily placed because of insufficient material. These are *Limonia Poggei*, var. *latialata* De Wild., doubtless distinct from *L. Poggei*, and *Limonia Demeusei* De Wild. Both have been described and beautifully figured.<sup>1</sup>

In addition to the material cited, specimens are to be found in the various European herbaria and in the National Herbarium at Washington, D. C., which it has been impossible to place, owing to the lack of flowers or fruits. This additional material represents collections, principally from Congo, by Auguste Chevalier, Ém. and M. Laurent, Demeuse, L. Gentil, and others.

#### POSSIBLE USES OF THE AFRICAN CHERRY ORANGES

The bringing to light of a new genus belonging to the true-orange group opens up a new field for the plant breeder, especially as some of the species are said to bear delicious fruits in abundance.

The unusually large compound leaves—often with five leaflets, each one of them larger than any ordinary orange leaf—give several of the species of *Citropsis* a distinct advantage over any other member of the true orange group. Large leaves are an outward and visible sign of an active assimilating system, and it must not be forgotten that over three-fourths of the dry substance of a plant is made up of starch, sugar, oil, flavoring matter, and other substances manufactured in the leaves, and a species with large leaves is equipped with the first essential for rapid growth and for developing sweet fruits of high flavor.

<sup>1</sup> Wildeman, Émile de. Études sur la Flore du Bas- et du Moyen-Congo. In Ann. Mus. du Congo, Bot. s. 5, v. 1, p. 159-160, pl. 51, 53. 1904.  
*Limonia Poggei*, var. *latialata*. In Gard. Chron., s. 3, v. 53, no. 1380, p. 378, fig. 159, June 7, 1913.



## GRAFTING OF CITROPSIS

Experiments conducted under the directions of the authors in the greenhouses of the Department of Agriculture at Washington, D. C., show that *Citropsis Schweinfurthii* can be grafted readily and that it will grow rapidly and vigorously on sweet orange, sour orange, grapefruit, and lemon stocks. It can also be grafted on the tabog (*Chaetosperrum glutinosa*) and the wood-apple (*Feronia elephantum*), two stocks on which species of *Citrus* graft readily. However, it does not grow as vigorously on these stocks as on *Citrus*. The very rapid growth of *Citropsis* when grafted on *Citrus* (see Pl. XLIX) is an added and striking proof of the close affinity of these two genera. Additional experiments in budding and grafting on other genera related to *Citrus* are now under way.

In view of the considerable botanical differences between *Citrus* and *Citropsis*, it is probable that the latter will show immunity to diseases and adaptations to soil and climatic conditions not possessed by the stocks upon which citrous fruits are commonly grafted. Experiments conducted by the authors have already indicated that *Citropsis Schweinfurthii* is well adapted to poor, sandy soils ("high pine lands") in Florida. Every new stock well adapted to *Citrus* gives the grower and the pathologist a new tool in the work of perfecting the culture of citrous fruits and in preventing the ravages of diseases by using stocks which are immune. The scarcity of material of the African cherry oranges has hitherto prevented any extensive experiments in the use of this new stock, but grapefruit and oranges have both been budded successfully on *Citropsis* stocks in the greenhouse at Washington and out of doors in Florida.

## HYBRIDIZATION OF CITROPSIS

The fact that there are a number of closely allied yet distinct species of *Citropsis* native to the forests of tropical Africa is an advantage to the plant breeder in furnishing material for the improvement of the African cherry oranges by hybridization. Whether the waxy-fruited species will yield edible hybrids when crossed with the juicy-fruited species can only be told by experiment.

So far, the scarcity of flowers of the African cherry oranges has prevented any decisive test as to whether they can be crossed with species of *Citrus* or not. This much can be said, that flowers of the common lime, *Citrus aurantifolia* (Christm.) Swing., pollinated with *Citropsis Schweinfurthii* set fruit and produced seed. Only a few seed were secured and none of them gave rise to a hybrid, but this is not uncommon in *Citrus*. The fact that the pollen of *Citropsis* was able to cause the development of seeds is a very hopeful sign that hybrids will be secured from pollinations in the course of the breeding experiments now being carried on by using the pollen of *Citropsis* on as many species of *Citrus* as possible.

That hybrids of the common citrous fruits with the African cherry oranges would be promising table fruits is rendered probable by the fact that both *Citrus* and *Citropsis* have species which in a wild state yield fruits beautiful to the eye, fragrant, and delicious to the taste.

Because of their beautiful foliage, their very fragrant, large white flowers, much resembling those of the orange or lime, and their abundant, though small, fruits, borne in tufts like cherries, the African cherry oranges are of unusual promise for ornamentals and for hedge plants in subtropical regions.

The fact that the true relationships of so large and so striking a group of plants, ranging clear across equatorial Africa, could remain misunderstood by botanists for so long a time, is another proof of the rich harvest of new material which awaits the attention of the plant breeder as soon as a critical taxonomic study of the wild relatives of our principal cultivated plants makes it available for his use.

PLATE XLIX. *Citropsis Schweinfurthii* grafted on grapefruit stock (*Citrus decumana*), showing vigorous growth made in  $2\frac{1}{2}$  years. Plant grown in greenhouse, Department of Agriculture, Washington, D. C., from seed from Budongo Forest, Uganda, Africa. (C. P. B. No. 2902.) One-sixth natural size.



# PRELIMINARY AND MINOR PAPERS

## WINTER SPRAYING WITH SOLUTIONS OF NITRATE OF SODA<sup>1</sup>

By W. S. BALLARD, *Pathologist, Fruit-Disease Investigations, Bureau of Plant Industry*, and W. H. VOLCK, *County Horticultural Commissioner of Santa Cruz County, California*.

### INTRODUCTION

Recently several investigators<sup>2</sup> have reported results in shortening the rest period of a number of woody plants by immersing the dormant shoots in weak nutrient solutions or by injecting solutions of alcohol, ether, and various acids into the twigs. These experiments have been conducted in the laboratory with short cuttings of the plants. The effect of such treatment has been to force the dormant buds out several days ahead of the normal opening period.

During the last two years the writers have obtained similar and additional results on a much larger scale by spraying dormant fruit trees with strong solutions of certain commercial fertilizers, especially nitrate of soda. Since these experiments have been conducted on the entire trees in the orchard, it has been possible to observe the effects throughout the whole season. The investigations have not yet been carried far enough to permit drawing any conclusions regarding the physiologic action of such spraying, but because of its practical value these preliminary results seem deserving of attention at this time.

### EXPERIMENTS IN 1912

In the course of the investigations of the writers on the control of apple powdery mildew in the Pajaro Valley, Cal., it became evident that the general vigor of the tree and the thriftiness of the foliage growth had much to do with the success of the summer spraying treatment for the control of the mildew, and after a number of experiments in applying plant-food materials to the foliage in the form of summer sprays, and after seeing that certain crude-oil emulsions used as dormant sprays had a marked effect in stimulating an increased vigor of the trees the following spring, it was decided to try the effect of a strong solution of nitrate of soda as a winter or dormant spray. Caustic potash (potash lye) was also added for the purpose of giving the spray an insecticide value. The mixture was prepared according to the following formula:

Nitrate of soda.....	50 pounds.
Caustic potash.....	7 pounds.
Water.....	50 gallons.

The experiment was conducted in a Yellow Bellflower apple orchard owned by Mr. O. D. Stoesser, of Watsonville, Cal. This orchard is

<sup>1</sup> These investigations were conducted in cooperation between the Office of Fruit-Disease Investigations of the Bureau of Plant Industry and the Office of the County Horticultural Commissioner of Santa Cruz County, located at Watsonville, Cal. The writers' names appear above in alphabetical order.

<sup>2</sup> See references to literature, p. 444.

situated about 5 miles from the ocean shore and is in a district that is more subject to ocean fogs and trade winds than is the main portion of the Pajaro Valley. It is a common characteristic of the numerous orchards of Yellow Bellflower apples of this particular district that they bloom abundantly, but set only a partial crop. The trees are on a deep sedimentary soil and grow well.

Seven 12-year-old trees were sprayed on February 2, 1912. The application was very thoroughly made, so that all of the small twigs were drenched. About 7 gallons of spray solution were applied to each tree. Adjoining this row on one side was a check row of seven trees which received no winter spraying, and on the other side were several rows of seven trees each which received various applications of crude-oil emulsions and soaps. For the purpose of gaining some idea of the effect of nitrate of soda used as a fertilizer, 50 pounds were applied as a surface dressing to one vigorous tree selected from the row adjoining the nitrate-sprayed row. This fertilizer was later plowed in and washed down by the rains.

#### EFFECTS ON BLOSSOMING AND ON THE FOLIAGE

Notes taken at the time the trees were coming out in the spring show the following results:

April 7, 1912. Trees in the row sprayed with nitrate of soda and lye are well in bloom, while those in the check row adjoining and in the remainder of the unsprayed orchard are showing only an occasional flower fully opened.

April 14, 1912. The relative advancement of the row sprayed with a solution of nitrate of soda and lye and the check plot is the same as noted on April 7. The nitrate-sprayed trees are nearly in full bloom, whereas comparatively few blossoms have opened on the check plot.

When the check row had reached full bloom, the row sprayed with a solution of nitrate of soda and lye was practically out of bloom.

Thus, the nitrate spraying advanced the blossoming time about two weeks ahead of the normal period. It is characteristic of the Yellow Bellflower variety of apples in the Pajaro Valley that the foliage buds come out early, so that by the time the full-bloom period is reached the trees are showing a considerable amount of young foliage. The nitrate spraying produced a change in this respect. While the flower buds were greatly stimulated in coming out, the foliage buds were not so much affected, and the result was that when the trees sprayed with a solution of nitrate of soda and lye were in full bloom and two weeks in advance of the check trees in that regard, their foliage condition was relatively nearer that of the check. Plate L shows the comparative stages of the nitrate-sprayed and the check trees at that time. A decided contrast will be seen in the relative advancement of the bloom on the tree sprayed with nitrate of soda (Pl. L, fig. 1) as compared with the check tree (Pl. L, fig. 2). This contrast is shown more in detail in Plate LI, in which figure 1 shows a branch from a nitrate-sprayed tree, while figure 2 shows one from a check tree. Both branches were collected on the same day. An examination of the figures in Plate L will show that the advancement of the foliage on the nitrate-sprayed tree is comparatively less marked than that of the bloom. This same condition is shown in detail in Plate LI, in which it will be seen that there is relatively little difference in the advancement of the foliage of the sprayed and unsprayed branches. Later in the spring, however, the effect on foliage growth became more pronounced, and the sprayed trees assumed a more vigorous, green appearance than the check trees. The single tree that received the 50 pounds of nitrate of soda applied to the soil showed no greater vigor than the check trees.

Both the row sprayed with nitrate of soda and the check row received summer sprayings directed toward the control of apple powdery mildew and of codling moth and various other insect pests. While the treatment of the two rows was not the same, there was no essential difference in the results—that is, the crop loss from codling moth and other insect pests did not exceed 1 per cent on either plat and there was no damage to the fruit from summer spraying. It is therefore evident that the difference which showed up in the crop production of the two rows must be attributed to the winter nitrate spraying.

#### CROP RESULTS

The check row of seven trees, which received no winter spraying but which was properly protected by summer sprayings, produced 8 loose boxes of fruit at picking time. On the other hand, the adjoining row, sprayed in February with the solution of nitrate of soda plus lye, produced a total of a little over 40 boxes. Thus, the winter nitrate spraying increased the crop production to fully five times that of the unsprayed row. Similar adjacent plats, which were winter-sprayed with various crude-oil emulsions and soap sprays, produced crops varying from 5 to 9 boxes per plat. The single tree which received the 50 pounds of nitrate of soda applied as a fertilizer gave no increased production, whereas none of the trees in the nitrate-sprayed row failed to respond.

Regarding the single, heavily fertilized tree, it might be stated that in addition to its showing no increase in production, the tree bloomed no earlier than normal, there was no improvement in the growth and no change in its general appearance throughout the growing season of 1912, and in the spring of 1913 it came out normally and not differently from the other trees in the same row, being one of the trees in a check plat. The tree is still in normal condition and shows no noticeable effect from the heavy fertilizing. The orchard is not irrigated, and the rainfall has been much less than normal during the last two years.

Attention might again be called to the conditions under which these results were obtained—namely, thrifty-growing trees in a deep residual soil and having the characteristic of blooming abundantly each year but setting only a shy crop. Even the 40 boxes produced by the nitrate spraying does not represent the full crop that such trees should bear, but the fourfold increase much more than paid for the cost of spraying, and the possibility remains of still further increasing that production by similar treatment in following years.

#### EXPERIMENTS IN 1913

The one small experiment on seven trees in 1912 did not furnish sufficient grounds for drawing any general conclusions as to the applicability of winter nitrate spraying, but the striking results obtained opened a wide field of inquiry. For instance, potash lye was added to the solution of nitrate of soda in the experiment of 1912, so the questions arise as to whether the lye was necessary and whether an acid medium would increase or decrease the effect of the nitrate of soda; also, would a weaker nitrate solution prove as effective and would other nitrogen-bearing fertilizer materials, such as lime nitrate, lime cyanamid, and sulphate of ammonia, give similar results? Following along this line it would be interesting to know what effect, if any, the other fertilizer elements,

potash and phosphoric acid, might have when applied as sprays, and finally, what results might be obtained from a similar application of other substances not ordinarily considered as having any particular fertilizer value.

Experiments intended to answer these and a number of other more or less important questions were started in February, 1913, in the same orchard in which the previous year's work was done. Eleven 13-year-old trees were used in each plat. A frost occurred at the time the fruit was setting which ruined the crop and made it impossible to obtain results in crop production. Data were obtained, however, on the effect of the various sprays on the blossoming of the trees in the spring, and the notes taken may be summarized as follows:

The plats sprayed with nitrate of soda at the rate of 1 pound to the gallon came into bloom earlier than the check trees, just as they had done in 1912. This effect was more marked in the cases in which lye was added to the nitrate solution than when the plain water solution was used—that is, the addition of lye in the proportion of 16 pounds of caustic soda in 100 gallons of spray solution increased the action of the nitrate of soda in bringing the trees out earlier. Caustic soda appeared to be just as effective as caustic potash. Nitrate of soda used at the rate of half a pound to the gallon, either with or without the addition of lye, was not nearly so effective as a solution of 1 pound to the gallon. A solution of one-fourth of a pound to the gallon, with lye added, had practically no effect. Nitrate of soda, at the rate of 1 pound to the gallon, to which oxalic acid was added in the proportion of 50 pounds to 125 gallons of solution, produced results similar to nitrate of soda plus lye, so far as the effect of hastening the blooming period is concerned. Lime nitrate, 130 pounds in 100 gallons of water, and lime cyanamid, 92 pounds in 100 gallons of water, stimulated an earlier blooming of the trees, and subsequent experiments will probably put these substances in a class with nitrate of soda. Normal Yellow Bellflower apple blossoms have considerable pink color, and it was interesting to note that when the trees sprayed with the lime cyanamid came into bloom the flowers were nearly white. The effects from sulphate of ammonia were not nearly so marked as those from nitrate of soda. These various nitrogen-bearing fertilizer substances were used in such strengths as to carry relatively the same quantities of nitrogen per gallon. Sulphate of potash had some effect in stimulating an early blooming, but double superphosphate did not. Of a number of other substances tried, common salt used at the rate of 68 pounds to 100 gallons of water produced a distinct effect.

It will be borne in mind that the above remarks apply simply to the effects of the various sprays in causing an earlier blooming of the trees, but since this early blooming was a striking characteristic of the nitrate-sprayed trees of 1912, which showed a fourfold increase in production, it seems permissible to conclude that this effect on the fruit buds is some criterion of what might have been expected in the way of crop increase had not the fruit been lost by frost.

The row of seven trees used in the nitrate experiment of 1912 was left unsprayed this last season for the purpose of determining whether the nitrate effect would continue to the second year. It was noticed that the fruit buds on these trees were particularly large and plump, and somewhat unexpectedly at blossoming time these trees came into bloom several days ahead of the check rows. The bloom came out very uniformly all over the trees, whereas ordinarily it is considerably delayed on the wind-

ward side. Also, the individual blossoms were conspicuously larger than those of any other plat, and, so far as could be judged at the time the frost occurred, a good crop was setting all over the trees. Thus, it appears that this effect of the nitrate of soda had continued over to the second year.

At present, all things considered, the best results have been obtained by using a mixture made up as follows:

Nitrate of soda.....	200 pounds.
Caustic soda.....	25 pounds.
Water.....	200 gallons.

In preparing this solution the required quantity of water was placed in the spray tank and the agitator started. When the water was in motion, the required weight of nitrate of soda was added gradually. Any large lumps were first broken up into pieces about the size of hen's eggs. The caustic soda was then added, and in about 15 minutes from the time the preparation was begun the mixture was ready for applying.

The trees were very thoroughly sprayed on all sides, so that all of the small twigs were drenched. The best results so far obtained have come from the spraying applied about the 1st of February. Of course, weather conditions must be taken into consideration. A rain immediately following the application will wash much of the material off of the trees, and it is probable that at least a week of clear weather should follow the spraying, in order to insure good results.

In all of this work on spraying a solution of nitrate of soda on the trees a considerable quantity fell to the ground, and the question will be raised as to whether the various effects observed have not been simply the result of the fertilizer action of the nitrate on the soil. About 7 gallons of the solution were used in spraying each tree, and if the whole of this had gone on the ground it would have amounted to about 7 pounds of nitrate of soda per tree. The single tree in 1912 that had the 50 pounds of nitrate applied to the soil therefore received over seven times the total quantity applied to any single sprayed tree. As has been previously stated, this single, excessively fertilized tree bloomed no earlier than normal, produced no increased crop, and showed no improvement in general vigor and appearance; whereas, none of the trees in the sprayed plat failed to respond in all of these particulars. Of course, this single tree test in the application of nitrate to the soil is too small an experiment to permit concluding positively that the effects that we have reported from the spraying experiments are of an entirely different nature and belong in a different category from those produced by the ordinary soil application of nitrate. A careful consideration of the results of ordinary orchard practice in fertilizing seems to make it plain that there is no similarity between them and the results from spraying. For instance, in the usual practice of applying nitrate of soda as a fertilizer to apple orchards in the region of Watsonville, Cal., a winter or early spring application does not force the bloom out 10 days or 2 weeks ahead of the normal opening period and has had no measurable effect in increasing the set of fruit that same year. The fact that the addition of caustic soda or oxalic acid to the nitrate spray augments these various effects further emphasizes the difference between the results from spraying and the ordinary results from the application of fertilizer. Caustic-soda solution alone applied as a spray has no effect on the time of blooming or the crop production.



## EXPERIMENTS OF GROWERS IN 1913

## YELLOW BELLFLOWER APPLES

During the past season a number of growers made more or less extensive tests of the spraying with nitrate of soda. An aggregate of several hundred acres of Yellow Bellflower apples was sprayed with nitrate of soda plus caustic soda, but practically all of this acreage was in the same district in which the writers' experiments were conducted, so the crop was lost by frost. It was noticeable during the past summer, however, that the foliage in such orchards as received very thorough winter nitrate sprayings had a better appearance than in years past, due apparently to the effect of the nitrate. One orchard, that of MacDonald & Sons, is located in a district that practically escaped frost damage, and the results obtained indicated a marked crop increase in consequence of the spraying. The entire orchard, with the exception of a few trees, was sprayed with various combinations of nitrate of soda and lye, and, while no exact data on the production of the unsprayed trees as compared with the rest of the orchard was obtained, the amount of fruit on the trees indicated that the spraying had produced a marked increase. This conclusion was more reliably substantiated by comparing the total orchard production this year with that of previous years.

## SWEET CHERRIES

Mr. A. W. Taite, of Watsonville, sprayed portions of two blocks of Napoleon (Royal Ann) cherries with nitrate of soda, 1 pound to the gallon, to which caustic soda was added at the rate of 25 pounds to 200 gallons. Unsprayed rows adjoining the sprayed ones were left in each block. In one case the sprayed trees were distinctly advanced over the check trees in coming into bloom. In both cases there was an increase in the foliage growth and a consequent improvement in the appearance of the trees. No effect on crop production could be noticed, though it is possible that treatment in successive years may bring such results.

## PEARS

For our observations on pears the writers are indebted chiefly to Mr. George Reed, of San Jose, who carried out extensive tests in the orchards of the J. Z. & G. H. Anderson Fruit Co. The spraying was done about the 1st of February and the following notes are taken largely from Mr. Reed's observations:

CLAIRGEAU.—Four rows of about 40 trees each were sprayed with commercial lime-sulphur solution (33° Baumé) diluted 1 to 9. Adjoining these were four rows sprayed with lime-sulphur solution diluted 1 to 9 and to which was added nitrate of soda at the rate of 1 pound to the gallon of the diluted spray. The rows sprayed with the combined solution of nitrate of soda and lime-sulphur came into bloom about a week ahead of those that received the lime-sulphur solution alone. The development of the fruit on these nitrate-lime-sulphur solution rows continued to show an advancement of about a week throughout half the growing season, and at picking time the fruit was greener and hung on better than that of the plain lime-sulphur-solution rows. Both plats bore a full crop, so there was no opportunity for observing any effect on production. The Clairgeau variety blooms early, and the further advancement due to nitrate spraying might result in frost injury in some localities. The fruit ordinarily has a habit of dropping off during the latter part of the growing season. This difficulty, however, was largely eliminated on the nitrate-sprayed rows.

**COMICE.**—The major portion of the block was sprayed with a plain water solution of nitrate of soda at the rate of 1 pound to the gallon. A small portion was sprayed with commercial lime-sulphur solution, diluted 1 to 9, with nitrate of soda added at the rate of 1 pound to the gallon of diluted spray. Through a misunderstanding the men doing the spraying left no check rows in this block, so that crop data could not be obtained. However, Mr. Reed's exact knowledge of the previous production of this block as a whole indicates that the marked increased production this last season was more than probably due to the nitrate spraying. The Comice is a relatively shy bearer, and a valuable pear commercially, so that any increased production that could be obtained by nitrate spraying would be much appreciated by the grower. One portion of the block that regularly produces less than the remainder gave a good crop this year, and it appeared that the addition of the lime-sulphur solution augmented the effect of the nitrate of soda just as the addition of lye has done in the experiments of the writers.

**GLOUT MORCEAU.**—A block of Glout Morceau pears was sprayed with the combination of lime-sulphur solution, diluted 1 to 9, plus nitrate of soda 1 pound to the gallon of diluted spray. This block had never produced a full crop, and while no unsprayed checks were left, the increased production this year would appear to be due to the nitrate spraying.

**WINTER NELIS.**—A block of Winter Nelis pears was sprayed with a solution of nitrate of soda 1 pound to the gallon of water. No lime-sulphur solution was added in this case. No check rows were left, and a frost destroyed a large percentage of the fruit after it had set. However, at that time the trees were carrying the largest crop they had ever produced, and again it would appear that the nitrate spraying had had a beneficial effect. The trees came into bloom about 10 days ahead of normal opening period.

#### DISCUSSION OF RESULTS AND SUMMARY

It is not the writers' intention to convey the impression that dormant spraying with nitrate solutions will solve the problem of shy bearing of fruit trees nor offer a more advisable method of applying nitrogen fertilizer. The purpose of this paper is simply to present the results as they now stand.

It is evident that, at least under certain conditions, some varieties of apples and pears that are more or less self-sterile may have their crop production materially increased by dormant spraying with solutions of nitrate of soda plus lye. The combination of a solution of nitrate of soda and lime-sulphur is apparently capable of bringing similar results.

Actual quantitative data on increased production from spraying with a solution of nitrate of soda are available from only one source, that of the first experiment on Yellow Bellflower apples in 1912. No production records were obtainable from the various tests made by growers during the season of 1913, but the one test on Yellow Bellflower apples and several others on pears indicate that such an increase had undoubtedly been brought about. It is considered that the growers' knowledge of the crops of the previous years as compared with that of this year furnishes a basis for conclusions that are at least corroborative.

That nitrate spraying of dormant trees will bring about an earlier blooming of certain varieties of fruit is a satisfactorily established fact, which has been demonstrated on Yellow Bellflower apples at Watsonville, Cal., and on various varieties of pears at San Jose, San Juan, and Suisun, Cal., during the past season. How generally this statement will apply to other varieties of apples and pears and in other localities remains to be determined. Results on stone fruits have not been as striking as those on pears and apples, but it is possible that stronger solutions, earlier spraying, or a repetition of the spraying in successive years may bring about such results.

The greater danger of injury from frost that might result from forcing trees into bloom earlier than normal would have to be taken into consideration in making practical use of nitrate spraying in winter.

Aside from the effect on crop production, there has also been a very noticeable improvement in the color, abundance, and vigor of the foliage, and it seems possible that nitrate spraying of dormant trees may be a valuable supplement to the ordinary fertilizer practices in obtaining quick results in orchards suffering from lack of nitrogen.

The writers will make no attempt at present to explain the peculiar effect of nitrate of soda in increasing the production of more or less self-sterile varieties of fruits, or in improving foliage growth. The similarity between the writers' results in forcing dormant buds by winter nitrate spraying and the results obtained by other investigators by treating cuttings with various weak solutions has been mentioned. In experiments of the writers, however, a more or less lasting effect on the vigor of the foliage and also some valuable results in increasing crop production have been obtained. It furthermore appears that the effects obtained by spraying with a solution of nitrate of soda may continue over to the second year, as shown by the original plat of 1912, which was left unsprayed in the winter of 1913.

The effects of the nitrate spraying seem to be proportional to the strength of the solution employed and the thoroughness with which it is applied. The addition of caustic soda materially increases this action.

#### LITERATURE

The following is a short list of some of the more recent literature on forcing the buds of dormant cuttings of woody plants.

JESSENKO, FRANZ.

Einige neue Verfahren die Ruheperiode der Holzgewächse abzukürzen. Ber. Deut. Bot. Gesell., Bd. 29, Heft 5, p. 273-284, pl. 12, 1911; Bd. 30, Heft 2, p. 81-93, pl. 3, 1912.

Über das Austreiben im Sommer entblätterter Bäume und Sträucher. Ber. Deut. Bot. Gesell., Bd. 30, Heft 4, p. 226-232, pl. 9. 1912.

LAKON, GEORG.

Die Beeinflussung der Winterruhe der Holzgewächse durch die Nährsalze. Ein neues Frühtreibeverfahren. Ztschr. f. Bot., Jahrg. 4, Heft 8, p. 561-582. 1912.

WEBER, F.

Über die Abkürzung der Ruheperiode der Holzgewächse durch Verletzung der Knospen, beziehungsweise Injektion derselben mit Wasser. Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Bd. 120, Abt. 1, Heft 3, p. 179-194, pl. 1. 1911.

PLATE L. Fig. 1.—Yellow Bellflower apple tree in full bloom on April 16, 1912, showing effect of spraying with a solution of nitrate of soda plus caustic potash on February 2 previous.

Fig. 2.—Unsprayed check tree for comparison with figure 1.

The illustrations are from photographs taken on the same day.





PLATE LI. Fig. 1.—A branch from a Yellow Bellflower tree in full bloom on April 10, 1913, showing the effect of spraying with a solution of nitrate of soda plus caustic soda on February 3 previous.

Fig. 2.—A branch from an unsprayed check tree for comparison with figure 1.

The illustrations are from photographs taken on the same day.

# JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. I

WASHINGTON, D. C., MARCH 25, 1914

No. 6

## TYLOSES: THEIR OCCURRENCE AND PRACTICAL SIGNIFICANCE IN SOME AMERICAN WOODS

By ELOISE GERRY,

*Microscopist, Forest-Products Laboratory, Forest Service*

### GENERAL DESCRIPTION OF TYLOSES

The large open pores or vessels conspicuous in hardwoods frequently become closed by growths called tyloses.<sup>1</sup> These growths render the wood practically impermeable to air and liquids. On the split surfaces of a wood such as white oak or pignut hickory the tyloses appear in the vessel channels as glistening cellular growths resembling masses of soap bubbles. (Pl. LII, fig. 1.) These masses are protrusions from the living parenchyma cells of the wood itself into adjacent vessel or tracheid cavities. They enter at the thin places or pits in the wall of the wood elements (see Pl. LII, figs. 2 and 3), and expand to a greater or less degree. In the softwoods (Pl. LVI, fig. 1) tyloses are relatively small, but in the hardwoods they frequently form bladderlike sacs of considerable size (Pl. LII, figs. 2 and 3, and Pl. LIII, figs. 1, 2, and 3), often developing simultaneously in many of the parenchyma cells surrounding the tube-like vessel cavities. (Pl. LII, fig. 3.) Under such circumstances, if growth is vigorous, the tylosal sacs, after pushing into the vessel cavity, grow together, completely filling it. In this way the ability of the vessel to conduct air or liquid is effectually checked. (Pl. LIII, figs. 1 and 2.) Sometimes, however, the tylosal growths do not entirely fill the vessel, and only a clogging action results.

The purpose of this study was to determine the occurrence of tyloses in the most important commercial species of native woods and their significance in relation to the adaptability of these woods to certain practical uses.

Observations were made not only of the presence or absence of tyloses in a species, but also of the extent and degree of development and the regions (sapwood or heartwood) where the growths are found.

<sup>1</sup> These growths received, in 1845, the name "Thylle" (tyloses) from a German botanist who signed as "Ungenannte," or "unknown," the paper discussing them. This writer is, however, believed by Boehm and Winkler to have been Frä. Hermine von Reichenbach. The name "Thylle" is derived from the Greek word *θύλλη*, meaning a purse or sack. The occurrence of tyloses was, however, noted as early as 1675 by Malpighi, in the drawing of a cross section of chestnut wood. They are also given the descriptive name "Füllzellen," or filling cells, by the Germans.



Only a brief discussion is given of the causes leading to the formation of tyloses or of their function in the living plant, since studies for this purpose have already been made by other investigators.

#### MORPHOLOGICAL RELATIONS OF TYLOSES IN WOODY TISSUE

##### ORIGIN AND DEVELOPMENT

A tylose can not be considered as a distinct cell, for as a rule a cell is defined as a body consisting of cell substance, cell wall, and cell nucleus. With very rare exceptions (Molisch)<sup>1</sup> a tylose, as found in woody tissue, is not completely surrounded by a wall and has no nucleus. It is only a portion or prolongation of a wood or medullary-ray parenchyma cell. (Pl. LII, figs. 2 and 3; Pl. LVII, fig. 2.) Frequently more than one tylose is formed from one parenchyma cell, but only one active nucleus—that of the parenchyma cell—is present, though this may be found in one of the tyloses. (Pl. LII, fig. 3.) A parenchyma cell which has given rise to two tyloses is shown in Plate LII, figure 2.

The growing or arching out of tyloses has been found to follow a reduction in internal pressure or cessation in sap conduction in the large vessels. When this occurs, the living parenchyma cells, which possess a considerable growth potential, expand and press into the adjacent empty vessel cavities. In pitted vessels this expansion is localized in the thin unlignified membranes of the one-sided bordered pits which are present on the dividing walls between vessels or tracheids, and parenchyma (De Bary; Green; Haberlandt; Hanausek; Molisch; Rees; Russow; Sachs; Strasburger; and Winckler). These membranes contain plasma and therefore possess the power of growth. The internal pressure of the turgid parenchyma cells, when exerted against these relatively thin spots or pits, causes the pit membranes to stretch and grow by intussusception<sup>2</sup> (Green; Molisch). The protrusions increase gradually in size and finally develop into the characteristic bladder-shaped sacs known as tyloses. An open passage through the space previously occupied by the unstretched closing membrane of the pit is formed in this way between the tylose and the parenchyma cell. (Pl. LII, fig. 2.) The contents of the tylose are therefore the same as those of the parenchyma cell.

##### NORMAL AND ABNORMAL TYLOSE FORMATION

It has been shown beyond doubt that the wounding of trees through cuts or bruises or at the points where branches are broken off tends to stimulate tylose formation, and throughout the study this mode of tylose formation has been constantly borne in mind. Generally, however, tyloses are not due to wounding. They are a characteristic feature of the normal uninjured wood of many families of trees. Nevertheless,

<sup>1</sup> Bibliographic citations in parentheses refer to "Literature cited," pp. 468-469.

<sup>2</sup> "Intussusception" means in botany, according to Nageli, the growth of cell walls by the irregular interposition of new solid particles between those already in existence.

the wood produced by felling the tree may have an important bearing on the presence of tyloses in the outer rings of a log, where the parenchyma cells are still living and capable of growth. It is possible to find in these rings young or old, or large and small, tyloses together in the same vessel. (Pl. LIV, R<sub>3</sub>.) Although exceptions have been noted, the idea that a considerable number of the outer rings are entirely free from tyloses has, however, been very generally accepted (Strasburger).<sup>1</sup> The data obtained from the present study show that there is a very considerable formation of tyloses in the outer rings of the sapwood. The question then arose as to whether these sapwood tyloses were of normal origin or whether they were due to some wound stimulus, such as the felling of the tree. It was finally concluded that they were normally formed tyloses, because their development throughout the vessels was very uniform instead of being sporadic or irregular, as in the case of tyloses associated with wounds (Pl. LIV, R<sub>1</sub> and R<sub>2</sub>), and because an examination of branches from living trees of *Rhus*, the sumach, *Catalpa*, and *Robinia*, the black locust, made immediately after cutting, confirmed the other observations of the relatively early formation of tyloses in many species. In material which was not received for examination until several weeks after it was cut, thin, irregularly distributed tyloses were often found in the outer vessels, though the latter must have been functioning in sap condition at the time the tree was felled.

It is noteworthy also that in this study tyloses were found to reach the most remarkable development in ring-porous woods, such as oak, hickory, black locust, or osage orange. (Pl. LIII, figs. 1 and 3, and Pl. LVI, fig. 2.) In woods where tyloses are few and scattered there is considerable variation from specimen to specimen in the actual number of tyloses present. This tendency is clearly shown in the woods of the diffuse porous group. (Table II.) It is also noticeable that in the two or three rings surrounding the pith in a diffuse porous wood tyloses are often much more abundant than elsewhere in either the heartwood or sapwood.

#### EFFECT OF THE DISTRIBUTION OF PARENCHYMA TISSUE

Since tylose formation depends upon the presence of parenchyma cells either in the form of wood parenchyma or medullary rays in close proximity to vessels or tracheids, the variation in position, abundance, and vitality of these cells affords at least a partial explanation of the irregular development of tyloses in different species of wood. Parenchyma tissue is considerably developed in the following families and their respective genera.<sup>2</sup> This study has shown that in these families are a large number of native woods exhibiting tyloses.

<sup>1</sup> Tyloses are . . . instrumental in closing the water courses of the heartwood. . . . These are intrusive growths from living cells which penetrate the cavities of the adjoining tracheal elements during the transition of sapwood into heartwood.

<sup>2</sup> Solereder, Hans. *Systematic Anatomy of the Dicotyledons* . . . v. 2, p. 1143. Oxford, 1908. Certain other woods with abundant parenchyma frequently produce gummy substances rather than tyloses.

Family.	Genera.
Cupuliferæ or Fagaceæ.....	Castanea, Fagus, Quercus.
Juglandaceæ.....	Hicoria Juglans.
Papilionaceæ.....	Robinia.
Magnoliaceæ.....	Liriodendron, Magnolia.
Moraceæ.....	Morus, Toxylon.

The arrangement of wood parenchyma cells in the annual ring has been divided <sup>1</sup> into three different types, as follows:

1. Terminal parenchyma, which is situated at the periphery of the annual growth ring, on the outer face of the summer wood.
2. Metatracheal or diffuse parenchyma, which is scattered among the other elements in the ring, usually forming tangential bands.
3. Paratracheal or vasicentric parenchyma, or parenchyma cells, aggregated around the vessels.

TABLE I.—*Native woods grouped according to the degree of tylose development and the most marked distribution of wood parenchyma in ring.*<sup>2</sup>

ABUNDANT TYLOSES.<sup>3</sup>

Species.	Type of parenchyma.	Species.	Type of parenchyma.
Catalpa speciosa.....	Paratracheal.	Hicoria orata.....	Paratracheal.
Chilopsis linearis.....	Do.	Juglans cinerea.....	Do.
Morus rubra.....	Do.	nigra.....	Do.
Rhus hirta.....	Do.	Quercus alba.....	Do.
Robinia pseudacacia.....	Do.	garryana.....	Do.
Toxylon pomiferum.....	Do.	lyrata.....	Do.
Hicoria alba.....	Do.	lobata.....	Do.
aquatica.....	Do.	macrocarpa.....	Do.
glabra.....	Do.	michauxii.....	Do.
lacinosa.....	Do.	minor.....	Do.
minima.....	Do.	platanoides.....	Do.
myristicaeformis.....	Do.	densiflora.....	Do.
odorata.....	Do.	marilandica.....	Do.

MANY TYLOSES.

Castanea dentata.....	Metatracheal.	Fraxinus lanceolata.....	Paratracheal.
Celtis occidentalis.....	Paratracheal.	profunda.....	Do.
Eucalyptus globulus.....	Do.	quadrangulata.....	Do.
Fagus atropunicea.....	Metatracheal.	Sassafras sassafras.....	Do.
Fraxinus americana.....	Paratracheal.		

SCATTERED TYLOSES.

Aesculus vetandra.....	Scanty para-tracheal.	Platanus occidentalis.....	Metatracheal.
Liquidambar styraciflua.....	Metatracheal.	Populus grandidentata.....	Terminal.
Liriodendron tulipifera.....	Terminal.	tremuloides.....	Do.
Magnolia acuminata.....	Do.	trichocarpa.....	Do.
fraseri.....	Do.	Ulmus alata.....	Paratracheal.
glauca.....	Do.	americana.....	Do.
		pubescens.....	Do.

<sup>1</sup> Jeffrey, E. C. A Natural Classification of Woods.

Holden, Ruth. Some features in the anatomy of the Sapindales. In Bot. Gaz., v. 53, no. 1, p. 50-58, pl. 2-3, 1912.

<sup>2</sup> The data here given concerning the distribution of parenchyma were obtained from: (1) Solereder, Hans, op. cit.; (2) Jeffrey, E. C., op. cit.; and (3) from original observations made during the study.

<sup>3</sup> By "abundant" is meant a very large number. "Many" is used to signify a considerable number but less than "abundant."

These three types of arrangement and the degree of their development bear a definite relation to the development of tyloses, since they indicate whether the parenchyma cells are near enough to the vessel cavities to send their prolongations into them. In addition to the wood parenchyma, the position and number of the medullary rays adjacent to the vessels must be taken into account. A grouping of the species of wood with the twofold object of indicating the distribution of tyloses and the arrangement of the wood parenchyma clearly brings out some of the reasons why tyloses are so much more abundant in certain woods than in others. Wherever the paratracheal or vasicentric type of parenchyma is well developed, the tendency for marked tylose formation, or else for gum production, is very noticeable. From Table I it is further evident that when tyloses are strongly developed either paratracheal or abundant metatracheal parenchyma is always found.

#### SHAPE, THICKNESS OF WALL, AND CONTENTS OF TYLOSES

The shape of the tylosal projections varies widely. They are sometimes spherical, or again they appear as elongated vesicles. (Pl. LII, fig. 3; and Pl. LIII, figs. 1, 2, and 3.) Often when the walls are very thin they appear much collapsed and wrinkled as, for instance, in ash or the wound tyloses in cow oak. (Pl. LIV, R1.) The extent to which the tylose wall increases in thickness varies also. The wall may be an extremely thin delicate membrane as found in ash or osage orange (Pl. LV, fig. 2) or it may be of medium thickness as in oak. (Pl. LIII, figs. 1 and 2.)

The contents of the tyloses are in general the same as those of the parenchyma cells producing them. Starch is common, and resin, calcium crystals, and gums have also been observed.

When normal parenchyma cells do not give rise to tyloses, the so-called "gums" (Prael)<sup>1</sup> are often produced, as in mesquite, maple, or cherry. This gum usually collects in the vessels (Pl. LIII, fig. 4) and parenchyma cells. In the vessels it sometimes assumes the form of globules or droplets which may easily be mistaken for tyloses. In order to determine whether gum or tyloses are present, a section of the wood may be treated with some gum solvent, such as absolute alcohol or caustic soda. When the wood is dry, the gum droplets are often characteristically cracked and split. Their general appearance is illustrated in Plate LIII, figure 4.

#### MATERIAL AND METHODS USED IN THE STUDY

The material used for this study of tyloses was a collection of logs of commercial size from native-grown trees. As a basis for the study of tyloses this material was unique, since most of the work of other investigators has been done not on wood from the bole of the tree, but on

<sup>1</sup> "Schutzgummi."

branches, twigs, roots, leaves, vines, herbaceous plants like the squash, or on such of the lower forms as ferns,<sup>1</sup> and did not cover to any extent the American species.

The method of examining the wood was as follows: The ends of the logs which form the collection of commercial American woods (Pl. LIX, fig. 1) of the Forest-Products Laboratory were examined with a hand lens. Blocks cut from these were also studied microscopically. Small strips extending from the bark through the trees to the pith, including the sapwood, the so-called transition region, and the heartwood,<sup>2</sup> were cut from the logs. Microtome sections about 1 inch by one-half inch in area and 5 to 20 micromillimeters in thickness were cut from the three planes, transverse, radial, and tangential, taken from each of these different regions and were studied under the compound microscope. The observations for hardwoods are given in Table II. Stains were often employed to differentiate the tissues, and macerations were made with potassium hydroxide or chromic acid for special studies of the relations between the tylose and the parenchyma cell producing it. Fresh material from seedlings and branches was also examined, in order to determine whether the sapwood tyloses were of normal or abnormal origin.

The Forest-Products Laboratory collection of woods begun in 1910 is not yet complete, and in many cases only one log of a species was available for study. Nevertheless, the majority of the commercially important species are included in the laboratory collection, and in addition to the study of these it was possible to make further observations on authentic material of a number of other important species. Moreover, whenever two or more specimens of the same species were examined, results were

<sup>1</sup> This list of the plant genera where tyloses have been found in wood, roots, leaves, or other portions is given by Küster. It includes Molisch's observations on the Vienna wood collection and other material as well as those of other authors, whose names are given in parentheses after the genera they investigated,

Abies (Raatz).	Coccoloba.	Laurus.	Portulacca.
Achyranthes.	Coleus.	Ligustrum.	Prunus (Wieler).
Aesculus (Maile, Tison).	Convolvulus (Dutailly).	Loranthus.	Pterocarya.
Alnus (Tison).	Cornus (Maile).	Loxapterygum.	Quercus.
Ampelopsis.	Corypha.	Machura.	Rhus.
Aralia.	Cucumis.	Mansoa.	Ricinus.
Aristolochia (Tison).	Cucurbita.	Maranta.	Robinia.
Artocarpus.	Cuspidaria.	Micania.	Rosa (Maile).
Arundo.	Dahlia.	Morus.	Rubia.
Asarum.	Diospyros.	Musa.	Rumex (Dutailly).
Banisteria.	Elacagnus.	Ochroma.	Salix.
Begonia.	Euphorbia.	Olea.	Sambucus.
Betula.	Fagus.	Ostrya.	Santalum.
Bignonia.	Ficus.	Passiflora.	Schinus.
Boehmeria.	Fraxinus.	Paulownia.	Sideroxylum.
Broussonetia.	Gleditsia (Tison).	Perilla.	Solanum.
Byronia.	Hammamelis (Tison).	Pharbitis.	Sparmannia.
Canna.	Hedera.	Philodendron.	Strelitzia.
Carica.	Hedychuim.	Phyllanthus.	Styinatophyllum.
Carya.	Heliconia.	Picea (Raatz).	Taraxacum.
Cassia.	Humulus (Tubouff).	Pinus (Raatz).	Thunbergia.
Castanea.	Inula.	Piratinera.	Ulmus.
Catalpa.	Jatropha.	Pistacia.	Urtica.
Celtis.	Juglans.	Plantago.	Vitis.
Chilantus.	Koelreuteria.	Platanus.	Xanthoxylon (Tison).
Cladrastis (Tison).	Latania.	Populus.	

<sup>2</sup> The cross section of a mature tree may be divided into at least two regions: The outer or last-formed rings, variable in number, which are termed the "sapwood" or "alburnum," and the inner rings around the pith or center of the tree, which in dry material are sometimes indistinguishable in appearance from sapwood, but which are more often definitely marked by a difference in color and are then termed the "heartwood" or "duramen." (Pl. LIX, fig. 1.)

found to check reasonably well, as shown in Table II. The greatest variation occurs in the species in which tyloses are very rare or else scatteringly developed and, therefore, where their practical importance is relatively slight.

#### OCCURRENCE OF TYLOSES IN NATIVE HARDWOODS

Table II gives the results of observations made on the distribution and region of first development of tyloses in 143 specimens of hardwoods grown in the United States. The very marked development of tyloses in certain species has been noted in Table I.

Special attention was given to the early development of tyloses. The results show their presence in the sapwood of all the species in which they occur in the heartwood. The hickories, for instance, give some interesting data concerning the occurrence of tyloses in sapwood. It has been maintained that if tyloses ever occurred in sapwood they would be found only in very narrow sapwood—that is, where the transition from sap to heartwood begins at the end of the first or second year after the ring is formed, as, for instance, in some of the oaks. In the hickories, however, tyloses are always present in the sapwood, and are generally developed even in the outermost rings as abundantly as in the heartwood. Plate LIII, figure 3, shows a cross section of the sapwood of pignut hickory (*Hicoria glabra*), including the fourth to the seventh rings in from the bark. This particular tree had 31 rings of sap, or uncolored wood, and tyloses were well developed in the very outermost rings. (Pl. LIX, fig. 1.)

Tyloses are normally lacking in the red-oak group, although there are many exceptions. An illustration of vessels not filled by tyloses is given by those in the middle of Plate LIV, *R*<sub>2</sub>, and by some of those in Plate LV, figure 1. In some cases tyloses occur in individual vessels in species ordinarily free from them, as Spanish oak. (Table II.) In several instances the few scattered tyloses present in both the sapwood and heartwood have a rather abnormal appearance and are associated with areas of fungous growth. (Table II, Scarlet oak.) In certain species of the red-oak group, however, as blackjack oak (*Quercus marilandica*), tyloses are very generally developed in both the sapwood and heartwood.

In the white oaks, in contrast to the red-oak group, tyloses are generally very abundant, even in the outermost rings. Some of the white oaks where tyloses are slow in forming show striking examples of the growth and development of the tylose in its early stages. This is illustrated in Plate LII, figure 3, which is a reproduction of a photomicrograph of a cross section of California white oak, or valley oak (*Quercus lobata*), showing a piece of the sapwood next to the bark. Fragments of the bark may be seen at the top of the illustration. The relatively small bladderlike cells here shown increase in size until they grow together and fill the vessels as shown at the bottom of this illustration and in Plates LIII, figures 1, 2, and 3, and LV, figure 2.

TABLE II.—Occurrence of tyloses in the large vessels of the hardwoods.<sup>1</sup>  
THE DIFFUSE POROUS WOODS.

Species.	Common name.	Tyloses in sapwood.			Tyloses in heartwood.
		Counting in from bark—		General character.	
		First appear in ring—	Full development in ring—		
<i>Acer macrophyllum</i> Pursh.	Oregon maple.			None present.	None present ("gum" frequently found).
<i>Acer negundo</i> L.	Box elder.			do.	Do.
<i>Acer rubrum</i> L.	Red or soft maple.			do.	Do.
<i>Acer saccharinum</i> L.	Silver maple.			do.	Do.
<i>Acer saccharum</i> Marsh.	Sugar or hard maple.			do.	Do.
<i>Acer saccharum nigrum</i> (Michx. f.) Britt.	Black maple.			do.	None present ("gum" abundant).
<i>Aesculus octandra</i> Marsh.	Yellow buckeye.			Very rare, scattered (Pl. LII, fig. 2).	Scattered (most near pith).
<i>Allanthus glandulosa</i> Desf.	Tree of heaven.			None present.	None present ("gum" frequently found).
<i>Alnus oregona</i> Nutt.	Red alder.			No material.	Do.
<i>Amelanchier</i> <sup>2</sup> <i>canadensis</i> (L.) Medic.	Service.			None present.	None present.
<i>Betula lenta</i> L.	Sweet birch.			do.	None present ("gum" frequently found).
<i>Betula lutea</i> Michx. f.	Yellow birch.			do.	Do.
<i>Betula nigra</i> L.	River birch.			do.	None present ("gum" sometimes found).
<i>Betula papyrifera</i> Marsh.	Paper birch.			do.	Do.
<i>Betula populifolia</i> Marsh.	White birch.			do.	Do.
<i>Carpinus caroliniana</i>	Blue beech.			do.	None present.
<i>Cornus florida</i> L.	Flowering dogwood.			do.	Do.
Do.	do.			do.	Do.
Do.	do.			do.	Do.
<i>Crataegus</i> <sup>2</sup> <i>coccinea</i> L.	Scarlet haw.			None present. (Rare exceptions near wounds.)	Same as in sapwood ("gum" abundant).
<i>Crataegus tomentosa</i> L.	Pear haw.			None present.	Do.
<i>Fagus atropurpurea</i> (Marsh.) Sudw.	Beech whiteheart.			Very rare.	Same as in sapwood.
Do.	Beech with red heartwood.			Few.	Many, vessels generally filled.
Do.	do.			Very rare.	Do.

	Witch-hazel.		None present.	None present.
<i>Hamamelis virginiana</i> L.			do.	None present.
<i>Ilex opaca</i> Ait.	American holly.		do.	Do.
<i>Kalmia latifolia</i> L.	Mountain laurel.		do.	Do.
<i>Liquidambar styraciflua</i> L.	Red gum.		Scattered; a few well-developed examples.	Scattered.
Do.	do.		Generally scattered throughout.	Same as in sapwood.
Do.	do.		No material.	Generally scattered throughout.
<i>Liriodendron tulipifera</i> L.	Yellow poplar.		Scattered.	Do.
Do.	do.		No material.	Few scattered.
<i>Magnolia acuminata</i> L.	Cucumber tree.		Scattered; frequent.	Scattered; frequent.
<i>Magnolia fraseri</i> Walt.	Fraser umbrella.		Very rare; scattered.	Do.
<i>Magnolia glauca</i> L.	Sweet magnolia.		Rare; scattered.	Do.
<i>Mohrodendron carolinum</i> (L.) Britt.	Silverbell tree.		None present.	None present.
<i>Nyssa sylvatica</i> Marsh.	Black gum.		do.	Do.
<i>Nyssa aquatica</i> L.	Water gum.		do.	Do.
<i>Oxydendrum arboreum</i>	Sourwood.		Few.	Scattered (numerous near pith).
<i>Platanus occidentalis</i> L.	Sycamore.		Scattered.	Scattered.
Do.	do.		Very rare.	Rare (almost entirely lacking except near pith).
Do.	do.		No material.	Frequent; scattered.
<i>Populus grandidentata</i> Michx.	Large-tooth aspen.		Numerous.	Many.
<i>Populus tremuloides</i> Michx.	Aspen.	2	Scattered.	Same as in sapwood (many near pith).
Do.	do.	1	Scattered (many in two outer rings).	Scattered.
Do.	do.		No material.	Scattered; numerous.
<i>Populus trichocarpa</i> Torr. and Gr.	Black cottonwood.		Scattered.	Scattered.
Do.	do.		No material.	Few.
<i>Prunus serotina</i> Ehrh.	Black cherry.		None present.	None present (abundant gum).
Do.	do.		do.	Do.
<i>Prunus pennsylvanica</i> L. f.	Wild red cherry.		do.	Do.
<i>Rhododendron maximum</i> L.	Great rhododendron.		do.	None present.
<i>Salix nigra</i> Marsh.	Black willow.	1	Numerous in four outer rings; scattered throughout.	Scattered.
Do.	do.		Very rare.	Very rare except in two rings near pith.
<i>Tilia americana</i> L.	Basswood.		None present.	None present.
Do.	do.		No material.	Do.

<sup>1</sup> Both the Latin and common names used are those given by G. B. Sudworth in Bulletin 17, Division of Forestry, Department of Agriculture, 1898, and in a later unpublished revision of the same.

<sup>2</sup> One of the Rosaceae, tyloses generally lacking in this family (Molisch).



TABLE II.—Occurrence of tyloses in the large vessels of the hardwoods—Continued.

## THE RING POROUS WOODS.

Species.	Common name.	Tyloses in sapwood.			Tyloses in heartwood.
		Counting in from bark—		General character.	
		First appear in ring—	Full development in ring—		
<i>Castanea dentata</i> (Marsh.) Borkh.	Chestnut.	1.	4.	Numerous; not exceedingly abundant.	Present throughout (rather thin walled).
Do.	do.			No material.	Abundant.
<i>Catalpa speciosa</i> Warder.	Hardy catalpa.	1.	2.	Abundant; thin walled.	Abundant throughout.
<i>Celtis occidentalis</i> L.	Hackberry.	Outer rings.		Scattered; well developed.	Numerous; slightly more than in sapwood.
Do.	do.			No sapwood material.	Numerous.
<i>Cercidium torreyanum</i> (Wats.) Sarg.	Palo Verde.			None present.	None present (abundant gum).
Do.	do.			do.	Do.
<i>Chilopsis linearis</i> (Cav.) Sweet.	Desert willow.	1.	3 on.	Abundant; thin walled.	Abundant throughout.
<i>Diospyros virginiana</i> L.	Persimmon.			None present.	None present.
<i>Eucalyptus globulus</i> Lab.	Blue gum.			Scattered; few.	Scattered.
<i>Fraxinus americana</i> L.	White ash.	1.	2 on.	Scattered; numerous; very thin-walled.	Numerous; same as in sapwood.
Do.	do.	2.	do.	Numerous; very thin-walled.	Numerous (vessels generally filled).
Do.	do.			No material.	Do.
<i>Fraxinus lanceolata</i> Borkh.	Green ash.	1.	2 on.	Abundant; very thin-walled.	Same as in sapwood.
<i>Fraxinus nigra</i> Marsh.	Black ash.			None present (much fungus in specimen).	None present (some "gum" present).
<i>Fraxinus oregana</i> Nutt.	Oregon ash.	1.		Very few; exceptionally; cases due to wounding in outermost ring.	Very few; thin; poorly developed.
Do.	do.			Outer sap lacking; many well developed but thin-walled.	No material.
<i>Proximus profunda</i> Bush.	Pumpkin ash.	1.	2 on.	Abundant; very thin-walled.	Same as in sapwood.
<i>Fraxinus quadrangulata</i> Michx.	Blue ash.	1.	do.	do.	Do.
<i>Gloditsia triacanthos</i> L.	Honey locust.			None present.	None present (gum frequently found).

Coffee tree.					
<i>Gymnocladus dioica</i> (L.) Koch.					
<i>Hicoria alba</i> (L.) Britt.					
Do.	2.	3 on.	Generally well developed.	Throughout as in sapwood.	
Do.	2.	do.	do.	Do.	
Do.	1 to 4 few.	5 on.	do.	Do.	
Do.	1 to 2 few.	3 on.	do.	Do.	
Do.	1.	8 on.	No material.	Throughout.	
<i>Hicoria aquatica</i> (Michx. f.) Britt.	1.	2 on.	Generally well developed.	Do.	
<i>Hicoria glabra</i> (Mill.) Britt.	2 to 3 few.	4 on.	Abundant throughout.	Same as in sapwood.	
Do.	1 to 3 least.	2 on.	do.	Do.	
Do.	1 to 4 least.	5 on.	Abundant throughout (exceptionally wide sapwood).	Do.	
Do.	1 to 3 least.	4 on.	Abundant throughout.	Do.	
<i>Hicoria laciniosa</i> (Michx. f.) Sarg.	1 to 3 least.	do.	Many.	Do.	
Do.	1.	2 on.	Generally well developed.	Do.	
<i>Hicoria minima</i> (Marsh.) Britt.	2 to 3 few.	3 on.	do.	Do.	
<i>Hicoria myristicifolius</i> (Michx. f.) Britt.	1.	2 on.	do.	Do.	
<i>Hicoria odorata</i> (Marsh.) Sarg.	1 to 3 least.	4 on.	do.	Do.	
<i>Hicoria ovata</i> (Mill.) Britt.	1 to 3 least.	2 on.	do.	Do.	
Do.	1 to 3 least.	4 on.	do.	Do.	
Do.	1 to 3 least.	2 on.	do.	Do.	
Five miscellaneous hickories.	Outer sapwood not finished.			Do.	
<i>Juglans cinerea</i> L.	1.	2 on.	Generally developed; many; most in 2 outer rings.	Many; full development.	
Do.	1.	do.	Generally developed; many; most in 7 outer rings.	Do.	
Do.	1.	1 on.	No material.	Do.	
<i>Juglans nigra</i> L.	1.		Abundant throughout.	Less strongly developed than in sapwood.	
Do.	2.	3 on.	No material.	Abundant.	
<i>Morus rubra</i> L.			Very abundant.	Same as in sapwood.	
<i>Prosopis juliflora</i> (Swartz) de C.			None present.	None present (abundant "gum").	
Erythrobalanus, the red oaks or black oaks.					
<i>Quercus rubra</i> L.			None present except as rare exceptions.	Same as in sapwood.	
Do.			do.	Do.	
<i>Quercus coccinea</i> Muenchh.	Abnormally numerous in 2 outer rings.		Scattered in third and fourth rings; generally absent. Fungus present; tyloses have abnormal appearance.	Do.	
Do.			None present.	None present.	
<i>Quercus digitata</i> (Marsh.) Sudw.			Very rare; one per ring to the inch.	Same as in sapwood.	
<i>Quercus imbricaria</i> Michx.			Scattered.	Do.	
<i>Quercus marilandica</i> Muenchh.	3.	3 on.	Strongly developed; full, large vessels.	Do.	

TABLE II.—Occurrence of tyloses in the large vessels of the hardwoods—Continued.

## THE RING POROUS WOODS—Continued.

Species.	Common name.	Tyloses in sapwood.			Tyloses in heartwood.
		Counting in from bark—		General character.	
		First appear in ring—	Full development in ring—		
Erythrobalanus the red oaks or black oaks—Continued.					
Quercus palustris Muenchh.	Pin oak			Extremely rare	Same as sapwood.
Do.	do.			None present	None present.
Quercus phellos L.	Willow oak			Very rare	Very rare.
Quercus velutina Lam.	Black or yellow oak	2	2 to 4 full	After fourth, scattered, frequent	Scattered.
Lepidobalanus, the white oaks:					
Quercus alba L.	White oak	1	3 on	Very numerous, young forms in outer rings.	Well developed throughout.
Do.	do.	1	3 on	do.	Do.
Do.	do.	1	3 on	Strong development	Abundant.
Do.	do.	2	2 on	do.	Do.
Do.	do.			No material	Many.
Quercus garryana Dougl.	Garry oak	2	2 on	Very abundant	Same as in sapwood.
Do.	do.			No material	Do.
Quercus lyrata Walt.	Overcup oak			Many; generally developed	Do.
Quercus lobata Nee.	Valley oak	1		Many; slow-forming, abundant young tyloses.	Abundant.
Quercus macrocarpa Michx.	Bur oak	1	1	Numerous to many; somewhat irregular.	Do.
Quercus michauxii Nutt.	Cow oak	1; many thin walled, 2; few or lacking.	3 on	Many	Do.
Quercus minor (Marsh.) Sarg.	Post oak	1		Abundant general development	Do.
Quercus platanoides (Lam.) Sudw.	Swamp white oak	1	3 on	Show young forms and gradual increase.	Do.
Quercus prinus L.	Chestnut oak			Rare exceptions.	Exceptions present.
Do.	do.			None present	None present.

Biotobalanus, the live oaks:							
Quercus densiflora Hook. and Arn	Tanbark oak					Scattered; thick walled	Same as in sapwood.
Do	do					Scattered	Abundant.
Quercus chrysolepis Liebm	Canyon live oak					Generally lacking	Same as in sapwood.
Rhus hirta (L.) Sudw	Shagbark sumach	1			1 on.	Abundant	Do.
Robinia pseudacacia L.	Black locust	1			1 on.	Abundant; all vessels crowded with thin-walled tyloses.	Do.
Do	do	1			2 on.	Abundant	Do.
Sassafras sassafras (L.) Karst.	Sassafras					Scattered; frequent	Do.
Toxylon pomiferum Raf	Osage orange	1			1 on.	Abundant; vessels crowded with thin-walled tyloses.	Do.
Ulmus alata Michx.	Wing elm					Numerous; scattered	Do.
Ulmus americana L.	White elm					Rare	Do.
Do	do					Generally scattered	Do.
Do	do					Rare; few	Do.
Ulmus pubescens Walt.	Slippery elm					Rare	Do.
Do	do					Few; scattered	Do.

## TYLOSES IN SOFTWOODS

Coniferous or softwoods lack the large open pores or vessels which characterize the hardwoods. They also either lack or show a scanty development of wood parenchyma, the chief source of tylose formation in the hardwoods. Since it is in relation to the closing of the vessels that tyloses are of practical significance, the study of tylose distribution in the conifers is of relatively small importance. However, since tyloses or tyloselike cells are often present in the tracheids or in the resin canals of certain normal coniferous woods, and since they have been found to play some part in penetration of wood preservatives and in resin flow, their occurrence in the softwoods was studied.

The occurrence of tyloses in coniferous woods has not received the attention given to their occurrence in hardwoods. Often their presence has been ignored, or they have been reported as entirely lacking.<sup>1</sup> When studied, moreover, investigations were usually confined to parts of the plant other than the wood,<sup>2</sup> though there are a few notable observations on their occurrence in the wood itself (Boehm; Chrysler; Conwentz; Küster; Mayr; Penhallow; Raatz).

## TRUE TYLOSES IN CONIFERS

Tyloses in normal coniferous wood arise chiefly from the parenchymatous cells of the medullary rays. (Pl. LVI, figs. 1 and 2.) As in the hardwoods, it is by the growth of the membranes of the one-sided bordered pits that tyloses are formed, especially where the pits are of large size, as in the white pines. In this case tyloses grow into the lumen of the tracheid, just as in hardwoods they grow into the vessels or pores. Tracheids, like vessels, function as sap conductors, but instead of having in their end walls actual openings of considerable size they have only relatively thin regions or pits. These are more or less completely closed by an irregularly thickened membrane, portions of which sometimes contain very minute perforations (Bailey). Thus in these elements already closed or nearly closed, tyloses have not the effect that they have in the open vessels of the hardwoods. Moreover, tylose formation of this type in conifers can only take place in a comparatively small percentage of the tracheids—that is, in those adjacent to the medullary-ray parenchyma cells produced as a result of wounds (Boehm; Raatz).<sup>3</sup>

## TYLOSELIKE CELLS IN THE RESIN CANALS

Aside from true tyloses, there is often observed in certain species of conifers a partial or complete closing of the resin canals, produced by parenchyma cells, but not by growth of the membrane of the one-sided

<sup>1</sup> Reported by Molisch after examining 700 species of plants of all sorts.

<sup>2</sup> They are said to be more abundant in the root than in the stem (Raatz). They also have been studied in the leaf and in the cone axis.

<sup>3</sup> Boehm and Raatz observed tyloses as a result of wounding in *Abies pectinata*, *Pinus sylvestris*, *Pinus strobus*, *Pinus excelsa*, *Larix europea*, and *Thuja occidentalis*.

bordered pit. Such growths are termed "tyloselike," since they produce an effect very similar to that produced by the true tyloses of the hardwoods.

Resin canals or ducts are normally present in the following coniferous genera: Larch, or tamarack (*Larix*), spruce (*Picea*), Douglas fir (*Pseudotsuga*), and pine (*Pinus*). These canals when seen in cross section often bear a superficial resemblance to the vessels or pores of the hardwoods. (Pl. LVII, fig. 1.) They are, however, different in both their origin and function. Resin ducts are not cellular elements, but simply intercellular spaces which result from the splitting apart of the common walls of a group of parenchyma cells. A very early stage of this splitting is shown in Plate LVI, figure 1. These parenchyma cells which surround the canal opening are called "epithelial cells." They are the seat of resin formation, and they cause the tyloselike closing of the resin canal. Certain of them often remain thin walled and contain plasma. (Pl. LVIII, figs. 2 and 5.) After they split apart to form the canal, when they change in shape and size, a further swelling and growth may take place which closes the canal entirely or in part. (Pls. LVII, figs. 1 and 2, and LVIII, figs. 2, 5, and 6.) The fact that it is the growth or expansion of the whole cell, and not a portion of the wall of that cell, together with a portion of the wall of the neighboring cell, as in the tylose-forming membrane of the one-sided bordered pits of the hardwoods, clearly indicates the difference between the true tyloses of the hardwoods and the tyloselike cells in the resin canals of the conifers.

#### OCCURRENCE OF TYLOSES AND TYLOSELIKE CELLS IN NATIVE CONIFERS

Over 600 permanently mounted sections from coniferous woods in the collection of the Forest-Products Laboratory were specially studied, while more than three times this number were examined unmounted.

#### TRUE TYLOSES

Ray or true tyloses were found in the normal wood of the conifers, but were not abundant. Their shape and general appearance are well illustrated in Plate LVI, figures 1 and 2. None of the long, saclike vesicles which sometimes fill the whole tracheid lumen in the roots of conifers were found. The greatest development of true tyloses was found in the soft pines. In this group they were better developed in spring wood than in summer wood and were more numerous in the sapwood than in the heartwood. Indeed, some of the pit membranes in the heartwood were concave in shape, appearing to have collapsed inward instead of protruding into the tracheid.

The size of the pits between the medullary ray cells and the tracheids in conifers bears a definite relation to the formation of tyloses. As a rule, the ray pits in the hard pines are small and tyloses are lacking,

Norway pine (*Pinus resinosa*), which is regarded as a hard or pitch pine, offers an exception to this. Here we find numerous tyloses, but here also we have large ray pits. The only soft pine examined which did not contain tyloses was piñon pine (*Pinus edulis*). This species is characterized by small ray pits instead of the large ones common to this group.

Of the other conifers all of the species listed below have small ray pits. No true tyloses were found in these species. (See Table III.)

TABLE III.—Occurrence of true tyloses in native conifers.

SOFT PINES.			
Species.	Number of specimens.	Sapwood.	Heartwood.
Limber pine ( <i>Pinus flexilis</i> ).....	1	Abundant..	
Sugar pine ( <i>Pinus lambertiana</i> ).....	1	...do.....	Numerous.
Western white pine ( <i>Pinus monticola</i> ).....	1	...do.....	Do.
White pine ( <i>Pinus strobus</i> ).....	2	Numerous..	Do.
Piñon pine ( <i>Pinus edulis</i> ).....	1	None.....	None.
HARD PINES.			
Norway pine ( <i>Pinus resinosa</i> ).....	2	Numerous..	Numerous.
Jack pine ( <i>Pinus divaricata</i> ).....	1	None.....	None.
Shortleaf pine ( <i>Pinus echinata</i> ).....	3	...do.....	Do.
Spruce pine ( <i>Pinus glabra</i> ).....	1	...do.....	Do.
Lodgepole pine ( <i>Pinus murrayana</i> ).....	1	...do.....	Do.
Longleaf pine ( <i>Pinus palustris</i> ).....	1	...do.....	Do.
Western yellow pine ( <i>Pinus ponderosa</i> ).....	2	...do.....	Do.
Pitch pine ( <i>Pinus rigida</i> ).....	1	...do.....	Do.
Loblolly pine ( <i>Pinus taeda</i> ).....	1	...do.....	Do.
Scrub pine ( <i>Pinus virginiana</i> ).....	1	...do.....	Do.
Table-mountain pine ( <i>Pinus pungens</i> ).....	1	...do.....	Do.
OTHER CONIFERS.			
Tamarack ( <i>Larix laricina</i> ).....	1	None.....	None.
Western larch ( <i>Larix occidentalis</i> ).....	1	...do.....	Do.
European larch ( <i>Larix larix</i> ).....	1	...do.....	Do.
White spruce ( <i>Picea canadensis</i> ).....	2	...do.....	Do.
Engelmann spruce ( <i>Picea engelmanni</i> ).....	1	...do.....	Do.
Black spruce ( <i>Picea mariana</i> ).....	1	...do.....	Do.
Red spruce ( <i>Picea rubens</i> ).....	2	...do.....	Do.
Sitka spruce ( <i>Picea sitchensis</i> ).....	2	...do.....	Do.
Douglas fir ( <i>Pseudotsuga taxifolia</i> ).....	2	...do.....	Do.
Balsam fir ( <i>Abies balsamea</i> ).....	2	...do.....	Do.
White fir ( <i>Abies concolor</i> ).....	1	...do.....	Do.
Lowland fir ( <i>Abies grandis</i> ).....	2	...do.....	Do.
Alpine fir ( <i>Abies lasiocarpa</i> ).....	1	...do.....	Do.
Red fir ( <i>Abies magnifica</i> ).....	1	...do.....	Do.
Noble fir ( <i>Abies nobilis</i> ).....	2	...do.....	Do.
Port Orford cedar ( <i>Chamaecyparis lawsonia</i> ).....	2	...do.....	Do.
Yellow cedar ( <i>Chamaecyparis nootkatensis</i> ).....	1	...do.....	Do.
California juniper ( <i>Juniperus californica</i> ).....	1	...do.....	Do.

TABLE III.—Occurrence of true tyloses in native conifers—Continued.

## OTHER CONIFERS—Continued.

Species.	Number of specimens.	Sapwood.	Heartwood.
Western juniper ( <i>Juniperus occidentalis</i> ).....	1	None.....	None.
Red cedar ( <i>Juniperus virginiana</i> ).....	1	...do.....	Do.
Incense cedar ( <i>Libocedrus decurrens</i> ).....	1	...do.....	Do.
Redwood ( <i>Sequoia sempervirens</i> ).....	1	...do.....	Do.
Bigtree ( <i>Sequoia washingtoniana</i> ).....	1	...do.....	Do.
Bald cypress ( <i>Taxodium distichum</i> ).....	1	...do.....	Do.
Yew ( <i>Taxus brevifolia</i> ).....	1	...do.....	Do.
Arborvitæ ( <i>Thuja occidentalis</i> ).....	1	...do.....	Do.
Western red cedar ( <i>Thuja plicata</i> ).....	1	...do.....	Do.
Eastern hemlock ( <i>Tsuga canadensis</i> ).....	1	...do.....	Do.
Western hemlock ( <i>Tsuga heterophylla</i> ).....	2	...do.....	Do.
Black hemlock ( <i>Tsuga mertensiana</i> ).....	1	...do.....	Do.

## TYLOSELIKE CELLS

The tyloselike epithelial cells which surround the resin canals were also carefully studied in *Pinus*, *Larix*, *Picea*, and *Pseudotsuga*. In these woods both the horizontal and vertical resin canals often contained distended cells which partly or sometimes completely filled the canal openings. (Pl. LVII, fig. 2; and Pl. LVIII, figs. 2, 5, and 6.) This closed condition of the vertical canals is particularly noticeable near the medullary rays. (Pl. LVI, fig. 1; and Pl. LVII, fig. 2.) The distended closing cells correspond to the plasma-containing cells described on page 446. (Pl. LVIII, figs. 2 and 5.) A large number of the canals were, however, entirely open.

In pines where many of the epithelial cells remain capable of growth, three types of conditions may be found in the canals.

(1) The canals of the sapwood, especially of the outermost ring, may not have yet opened—that is, the space which the canal will occupy may still be filled by the parenchyma cells which later form the epithelium. (Pl. LVI, fig. 1.)

(2) Many canals may be partly open. (Pl. LVII, fig. 1.) Frequently the cells surrounding the opening are somewhat contracted and collapsed; or, again, individual cells containing plasma may become distended, bow out into the open lumen of the canal, and thus assist in partially closing it.

(3) Canals in the heartwood as well as in the outer rings of the sapwood may be completely closed.<sup>1</sup> This may come about in two ways: First, the groups of parenchyma cells observed in the sapwood may

<sup>1</sup> Compare Thomson, R. B.



never have split apart to form a canal opening. This was demonstrated by the writer by means of serial sections following the course of a number of horizontal resin canals from the bark into the heartwood. Second, the canals once open may be closed completely by the growth of certain of the epithelial cells, as before explained. This closing is not produced by the equal action of all the cells which first split apart to form the canal, but only by the later growth of certain of these which possessed plasma and the growth potential for a longer period than their neighbors. (Pl. LVIII, fig. 5.)<sup>1</sup>

#### PRACTICAL SIGNIFICANCE OF TYLOSES

##### TYLOSES AS A NATURAL "FILLER"

A good instance of the part played by tyloses in the structure of wood is in the case of red oak and white oak. These two species have practically the same structure, yet the red oak can not be used for tight cooperage stock because the vessels are open tubes through which air or liquid can escape. (Pl. LIV, middle.) In white oak the vessels are completely closed by tyloses, as shown in Plate LIII, figures 1 and 2, or Plate LIV, R3.

In cabinetmaker's parlance, tyloses behave to some extent like a natural "filler." On a radial-cut surface the large vessels in the spring wood of a red oak appear like hollow grooves, while those in the white oaks are partly filled by the network of the tylosal cells which catch and hold paint, for example. (Pl. LII, fig. 1; and Pl. LIII, fig. 2.)

##### TYLOSES A FACTOR IN DURABILITY

It is of interest to note the presence of tyloses (or sometimes of gums) in the large vessels of those hardwoods which are particularly valued for their durability. Many factors, such as the chemical composition of the wood, its rate of growth, and hardness, are, of course, important in determining durability, but the effect of tyloses should not be disregarded. Moreover the vigorous growth of parenchyma, which in some cases manifests itself by causing tylose formation and in others by producing tannins, essential oils, etc., appears to be a fundamental characteristic of naturally durable woods. White oak, in which tyloses are abundant, is, for example, more durable than red oak, in which they are almost wholly absent. The tylose walls present an added obstruction to the advance of fungous hyphæ and tend to make the vessels impenetrable to air and water. They are especially effective in woods that have been dried.

Although sapwood contains tyloses, it is usually less durable than heartwood. The latter fact, however, holds true also for woods without tyloses and can probably be explained by the condition of such materials

<sup>1</sup> The illustrations reproduced in Pl. LVIII of all conditions of open and closed horizontal resin canals were taken from sapwood material.

in the sapwood as starches, which undergo a transformation when the heartwood is formed.

The following tabulation of the "Relative durability of hardwoods," compiled from the results of experiments, indicate that tyloses are a factor in durability. The more durable species will be found, with a few exceptions, to contain many or very abundantly developed tyloses. (See Tables I and II.)

RELATIVE DURABILITY OF HARDWOODS <sup>1</sup>*Durable.*

Black locust.	Chestnut.	White oak.	Cherry.
Catalpa.	Black walnut.	Post oak.	Persimmon.
Osage orange.	Live oak.	Black ash.	Slippery elm.
Mulberry.	Sassafras.	Honey locust.	Bur oak.

*Fairly durable.*

Yellow poplar.	Red oak.	Scarlet oak.	Butternut.
Red ash.			

*Not durable.*

Cottonwood.	Black oak.	Black gum.	Gray birch.
White elm.	Red birch.	Watergum.	Paper birch.
Red gum.	Beech.	Basswood.	Aspen.
Hard maple.	Hickory.	Buckeye.	Willow.
White ash.	Cucumber.	Sycamore.	

The results of tests on 30,160 fence posts <sup>2</sup> indicated the following untreated hardwoods, in order of their durability, as the most suitable: Osage orange, locust, mulberry, catalpa, certain oak (species not given), and black walnut. The length of life in service varied from 10 to 50 years.

Some observations <sup>3</sup> on the life of untreated hardwood railroad ties further confirm the relation between tyloses and durability. It must be borne in mind, however, that for this type of service hardness has been considered in judging durability. The list of woods, together with their life in years under traffic, is as follows:

Species.	Years of service.	Species.	Years of service.
Butternut .....	<sup>4</sup> Few.	Black walnut .....	9
Beech .....	Do.	Chestnut .....	5 to 10
Black, red, or yellow oak .....	4 to 5	Hickory .....	7 to 10
Post oak .....	6 to 8	Black locust .....	7 to 10
Sassafras .....	6 to 8	White oak .....	5 to 12
Chestnut oak .....	9	Mulberry .....	<sup>5</sup> Many.
Bur oak .....	9	Catalpa .....	Do.

<sup>1</sup> This list is offered to show the comparative durability of some American timbers. It is not presumed to obtain for all conditions.

<sup>2</sup> Crumley, J. J. The relative durability of post timbers. Ohio Agr. Expt. Sta. Bul. 219, p. 605-640, 10 pl. 1910.

<sup>3</sup> Tratman, E. E. R. Report on the use of metal railroad ties and on preservative processes and metal tie-plates for wooden ties. U. S. Dept. Agr., Div. For. Bul. 9, p. 216. 1894.

<sup>4</sup> Life not given.

<sup>5</sup> Little used.

A few exceptions are noticeable. Chestnut oak, for example, has very few tyloses, but is hard and strong. Butternut has many tyloses, but it is also much softer than the oaks. Hickory has many tyloses and is here considered as durable a wood as black walnut. This is contrary to observations of its durability by other investigators. The kind of beech used is not specified, but if it was "white-heart" beech tyloses were not present. The "red-heart" beech, which contains tyloses, is often reported as a very durable wood.

The following recent estimates are based on experience and actual inspection by the Forest-Products Laboratory of woods in service (Table IV):

TABLE IV.—*Life of untreated wood placed subject to decay.*

Untreated material.	Years.	Untreated material.	Years.
<i>Tyloses abundant or many; well developed.</i>		<i>Tyloses lacking or scattered; few or weakly developed—Contd.</i>	
Lumber:		Lumber—Continued.	
Chestnut.....	12	Maple.....	4
White oak.....	8	Birch.....	4
Posts:		Poplar.....	4
Locust.....	25	Cottonwood.....	4
Osage orange.....	40	Tupelo.....	4
Mulberry.....	20	Basswood.....	4
Catalpa.....	14	White-heart beech.....	4
Chestnut.....	10	Red gum.....	4
White oak.....	8	Sycamore.....	3
Ties:		Posts:	
Black locust.....	20	Red oak.....	5
White oak.....	8	Ash.....	5
Chestnut.....	7	Aspen.....	5
<i>Tyloses lacking or scattered; few or weakly developed.</i>		Gum.....	3
Lumber:		Ties:	
Elm.....	7	White-heart beech.....	4
Ash.....	5	Birch.....	4
		Maple.....	4
		Red oak.....	4
		Gum.....	3

#### TYLOSES A FACTOR IN CREOSOTE PENETRATION

##### EXPERIMENTS WITH HARDWOODS

The study of the effect of structure on the penetration of artificial preservatives, such as creosote, is a separate problem. Preliminary work has shown some interesting results concerning the treatment of certain tylose-filled hardwoods. A piece of air-dry black locust (*Robinia pseudacacia*), 9 by 1½ by 1 inch, was subjected to a thorough treatment with creosote in a treating cylinder. The piece contained sapwood and heartwood, the vessels of both of which were filled with tyloses. The stick when split open after treatment showed no penetration except a faint discoloration in the outer one-fourth inch of sap, which apparently did not extend to the tyloses filling the vessels, but was located only in a few scattered groups of fibers. The failure of the wood to absorb creosote

was not entirely due to the presence of tyloses, but the fact that the creosote did not penetrate the tylose-filled vessels is significant.

In a piece of desert willow (*Chilopsis linearis*), 4 by 1½ by 2 inches, treated with carbolineum, no penetration was visible in the heartwood except about one thirty-second of an inch near the surface. In the sapwood, however, where, as shown in Table I, the large vessels of the two outer growth rings are without tyloses, the dark discoloration of the preservative was clearly visible following the lines of these open vessels.

Sapwood in general absorbs creosote much more easily than heartwood. The supposed absence of tyloses in this region of the tree has previously been regarded as one reason for this fact. As soon, therefore, as it was satisfactorily determined that tyloses were unmistakably present in the sapwood, special experiments were undertaken to discover what effect they had on the absorption of the creosote. A piece of white oak was given a commercial treatment at the same time and under the same conditions as the black locust. The sapwood absorbed the oil fully, but the penetration stopped abruptly at the line of color demarkation between the sapwood and heartwood. (Pl. LIX, fig. 2, B.) To the eye the heartwood, except for a surface coating, was absolutely untreated. The vessels in both the sapwood and heartwood of this piece were filled with strongly developed tyloses. Microscopic examination showed that the tyloses in the vessels of the treated sapwood were entirely uncolored and exactly like those in the vessels of the heart which was untreated throughout. The tyloses had then effectually kept the creosote out of the vessels, although there had been a full treatment of the wood fibers of the sapwood. This shows that a considerable quantity of the preservative was absorbed in spite of the fact that the presence of tyloses kept the creosote out of the vessels. Hence, tyloses of themselves need not be regarded as preventing the possibility of treating this species, at least in the sapwood.

A piece of oven-dried hickory, 2½ by 2½ by 14 inches, made up of both heartwood and sapwood, was treated at the same time and under the same conditions as the oak and locust, and showed a thoroughly good penetration throughout. (Pl. LIX, fig. 2, C.) Nevertheless, when the wood was split, the tyloses, which were abundantly developed in the vessels of both the sapwood and heartwood, were white and unstained by the creosote, showing a marked contrast to the dark-brown fibers of the surrounding treated wood. (Pl. LII, fig. 1.)

The preliminary observations just described concerning the penetration of creosote were based on results of treatments made on single specimens of the species studied and were regarded rather as valuable indications than as conclusive evidence. To check them with other results, the treatments with creosote were repeated on other specimens of the woods previously used and more specimens of another species containing many tyloses. First, a piece of hickory taken from miscellaneous material was given a high-pressure treatment with creosote.

A good absorption was obtained in both the sapwood and heartwood. Nevertheless, the tyloses, which were everywhere well developed and undamaged in the large vessels of both regions, remained colorless and untreated. In addition, two other blocks of hickory from material collected with special care were also given pressure treatments in the cylinder. These specimens were from pignut hickory, *Hicoria glabra*, and mockernut hickory, *Hicoria alba*. Both specimens contained sapwood and heartwood, with tyloses strongly developed in the large vessels. Again, the wood was thoroughly treated with creosote in both the sapwood and the heartwood, and once more the tyloses could be observed on a split surface to be quite uncolored and visible even to the naked eye through their marked contrast with the blackish brown of the treated wood. (Pl. LII, fig. 1.)

Thus, results from four specimens of hickory from different sources clearly showed that in spite of the presence of tyloses a high absorption of creosote may be obtained in the wood substance outside of the vessels and the tyloses filling them.

The other species used in these experiments was the so-called red-heart beech, a form of *Fagus atropunicea*. This had white tylose-free sapwood, but a reddish heartwood with many tyloses. It was treated in the cylinder at the same time as some of the hickories. The sapwood was thoroughly penetrated, but the heartwood remained untreated except for a surface coating and a very slight infiltration near the ends.

Lastly, a second piece of white oak was treated, as a check on the piece treated previously. After the creosote treatment, which was given at the same time as that of the hickories and beech, the sapwood was found to be penetrated, and, as before, the heartwood was unpenetrated. Careful examination showed, however, that the discoloration of the creosote extended down the large vessels of the sapwood and into the tyloses which they contained. This apparent contradiction of previous observations was explained when the material was examined under the microscope. The tyloses were found to be full of fungous mycelium and riddled with holes produced by the hyphæ in passing through the tylose walls. Under these circumstances, even when abundant tyloses are present, it is clear that some penetration may be secured in the vessels.

The marked difference to be observed in the penetrance of creosote in treatments of red oak and white oak is, however, chiefly the result of the presence or absence of tyloses. The unobstructed vessels of red oak give such open channels and offer so much additional surface for absorption through their walls that the penetrability of the other elements lying between the vessels is of relatively little importance. In white oak, on the other hand, it is only the elements of structure other than the large vessels that are available for penetration. The type of penetrance obtained in red oak is shown in Plate LIX, figure 2, A. The dark streaks mark the course of the creosote, which passed almost entirely

through the open vessels. The practical effect of this is evident in the results obtained in penetrance treatments. It is possible to force creosote for long distances through red oak just as it would be possible to force it through similar distances in small open pipe lines. In comparison with this, the distance the oil will pass through white oak is very short, since it has to penetrate through many cell walls, and the resistance of the material must be overcome by high pressures.

Thus, although tyloses have a distinct effect, they are not the only factor in the penetrance of wood. The characteristics of the other elements in the annual ring must be considered. However, in the cases examined, wherever the large vessels contained abundantly developed tyloses or filling cells, the vessels and the tyloses, but not necessarily the rest of the woody tissues, were impenetrable to creosote.

#### OBSERVATIONS ON CONIFERS

The presence of resin canals and their condition—that is, whether they are open or partly or entirely closed by cells—considered in conjunction with the general permeability of the tracheids, is a factor of practical significance in the selection of wood for creosoting. (Pls. LVI and LVII.) The number of the resin canals is very small in comparison with the number of tracheids. However, if the canals are unobstructed, penetrance is easily obtained for considerable distances through their cavities. In a wood whose tracheids are penetrated with difficulty, the creosote does not spread to any great extent from the canals into the tracheids, even when the former are full. Nevertheless, the presence of creosote or other toxic liquid in the resin-canal regions, which are among the first affected by fungous infection, is of considerable assistance in prolonging the life of the wood. Many of the resin canals, especially the vertical canals in both the sapwood and the heartwood of the pines, are not completely closed (Pl. LVII, fig. 1, and Pl. LVIII, figs. 1 and 4) and can for this reason be penetrated. The effect of the presence or absence of tylose-like cells in the resin canals, while a minor factor, is significant in connection with the treatment of poles, ties, and paving blocks.

#### EFFECT OF TYLOSES ON THE WATER-LOGGING OF WOOD

In order to test the effect of tyloses on the water-logging of wood, some roughly comparable air-dry blocks of several species were placed in a tank of water and the length of time required to water-log each block sufficiently to sink it was noted. The blocks were grouped with reference to their specific gravity (dry)<sup>1</sup> and their actual weight. The woods in which tyloses were few or wholly lacking invariably sank before those containing abundant tyloses. Chestnut oak sank before white oak and bur oak, persimmon before osage orange, flowering dogwood before hickory, yellow poplar and aspen before catalpa, and blue beech and honey locust

<sup>1</sup>Sargent, C. S. Report on the Forests of North America . . . 612 p., maps. Washington, 1884. (U. S. 10th Census Reports, v. 9.)

before black locust. The dogwood and persimmon sank in about 18 hours, while the catalpa floated for 20 days, and one piece of black locust with a large percentage of heartwood remained floating for 46 days.

#### SUMMARY

The 143 specimens of hardwoods examined included 45 genera (94 species), of which 24 contained tyloses. The 60 specimens of conifers examined included 13 genera (45 species), of which 1 contained tyloses. Of the 139 species examined, 56, belonging to 25 genera, contained tyloses.

Tyloses were found in the sapwood of all species in which they occurred in the heartwood.

Well-developed tyloses were found in the outermost rings near the bark of 30 species of hardwoods.

True tyloses occur in the wood tracheids of certain pines, principally of the white-pine group.

Epithelial cells sometimes effect a partial or even complete tyloselike closing of the resin canals in *Pinus*, *Larix*, *Picea*, and *Pseudotsuga*.

A considerable proportion of the vertical canals, even in the heartwood of the pines, are fully or partly open.

Tyloses act like a natural filler in the hardwoods.

The woods in which tyloses are abundant as a rule are durable.

Tyloses, because they are very impermeable to air, water, and creosote, reduce the penetrance of the woods in which they are strongly developed. The presence of tyloses in the vessels of a hardwood, however, does not prevent the penetrance of creosote into the other wood elements.

#### LITERATURE CITED

BAILEY, I. W.

1913. Preservative treatment of wood. *In* Forestry Quart., v. 11, no. 1, p. 5-20, 2 pl.

BARY, ANTON DE.

1884. Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns. Translated by F. O. Bower and D. H. Scott ... p. 170. Oxford.

BOEHM, JOSEF.

1867. Ueber Function und Genesis der Zellen in den Gefassen des Holzes. *In* Sitzungsber. K. Akad. Wiss. [Vienna], Math. Naturw. Cl., Abt. 2, Bd. 55, p. 851-866, 2 pl.
1877. Ueber den aufsteigenden Saftstrom und den Abschluss lebender Zellen gegen äussere Einwirkungen. *In* Bot. Ztg., Jahrg. 35, No. 7, p. 112-113.
1879. Ueber die Function der vegetabilischen Gefässe. *In* Bot. Ztg., Jahrg. 37, No. 15, p. 225-239; No. 16, p. 241-258.

CHRYSLER, M. A.

1908. Tyloses in tracheids of conifers. *In* New Phytol., v. 7, no. 8, p. 198-204, pl. 5.

HABERLANDT, G. F. J.

1884. Physiologische Pflanzenanatomie. p. 217. Leipzig.
1887. Ueber die Beziehungen zwischen Function und Lage des Zellkernes bei den Pflanzen. p. 71-74. Jena.

## HANAUSEK, T. F.

1907. *Microscopy of Technical Products*. Translated by A. L. Winton and Kate G. Barber. p. 200. New York.

## KÜSTER, ERNST.

1903. *Pathologische Pflanzenanatomie*. 312 p., illus. Jena.

## MAYR, HEINRICH.

1883. Über die Vertheilung des Harzes in unseren wichtigsten Nadelholzbäumen. *In* Flora, Jahrg. 66 (n. R. Jahrg. 41), No. 14, p. 223.  
 1884. Entstehung und Vertheilung der Secretions-Organen der Fichte und Lärche. *In* Bot. Centbl., Bd. 20, No. 8, p. 246-253; No. 9, p. 278-283; No. 10, p. 308-310, pl. 1-3.  
 1893. Das Harz der deutschen Nadelwaldbäume. *In* Ztschr. Forst u. Jagdw., Bd. 25, p. 313-324, 389-417, 565-593, 654-670, pl. 1-2. Reprinted as Das Harz des Nadelhölzer ... 1894.

## MOLISCH, HANS.

1888. Zur Kenntniss der Thyllen, nebst Beobachtungen über Wundheilung in der Pflanze. *In* Sitzungsber. K. Akad. Wiss. [Vienna], Math. Naturw. Cl., Abt. 1, Bd. 97, Heft 6, p. 264-298, 2 pl.

## PENHALLOW, D. P.

1907. *Manual of the North American Gymnosperms* ... 374 p., illus. Boston.

## PRAËL, EDMUND.

1888. Vergleichende Untersuchungen über Schutz- und Kern-Holz der Laubbäume. *In* Jahrb. Wiss. Bot., Bd. 19, p. 1-81, pl. 1.

## RAATZ, WILHELM.

1892. Ueber Thyllenbildungen in den Tracheiden der Coniferenhölzer. *In* Ber. Deut. Bot. Gesell., Bd. 10, p. 183-192.

## REESS, MAX

1868. Zur Kritik der Böhm'schen Ansicht über die Entwicklungsgeschichte und Function der Thyllen. *In* Bot. Ztg., Jahrg. 26, No. 1, p. 1-11, pl. 1.

1896. *Lehrbuch der Botanik*. p. 88. Stuttgart.

## RUSSOW, EDMUND.

1872. Vergleichende Untersuchungen ... der Leitbündel-Kryptogamen, mit Berücksichtigung der Histologie der Phanerogamen ... 207 p., 11 pl. St. Pétersbourg. (Mém. Acad. Imp. Sci. St.-Pétersb., s. 7, t. 19, no. 1.)  
 1883. Zur Kenntniss des Holzes, insonderheit des Coniferenholzes. *In* Bot. Centbl., Bd. 13, No. 4, p. 134-144; No. 5, p. 166-173, pl. 1-5.

## SACHS, JULIUS.

1887. *Lectures on the Physiology of Plants*. Translated by H. M. Ward. p. 581. Oxford.

## STRASBURGER, EDUARD.

1891. Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. p. 191. Jena. (*His Histologische Beiträge*, Heft 3.)

1902. *Das botanische Practicum* ... Aufl. 4, p. 249. Jena.

## —, SCHENCK, HEINRICH, NOLL, FRITZ, and KARSTEN, GEORGE.

1908. *Text-Book of Botany*. ed. 3, rev. with German ed. 8, 746 p., illus. London.

## THOMSON, R. B.

1913. On the comparative anatomy and affinities of the Araucarineae. *In* Phil. Trans. Roy. Soc. London, s. B, v. 204, p. 1-50, pl. 1-7.

## WINCKLER, HANS.

1905. Ueber einen neuen Thyllentypus nebst Bemerkungen über die Ursachen der Thyllenbildung. *In* Ann. Jard. Bot. Buitenzorg, v. 20 (s. 2, v. 5), pt. 1, p. 19-37.

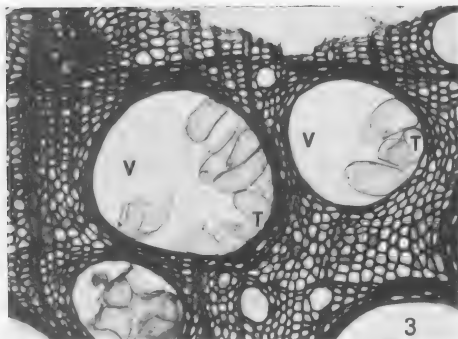
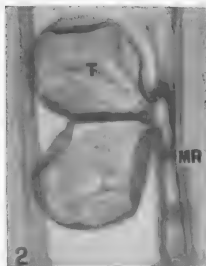
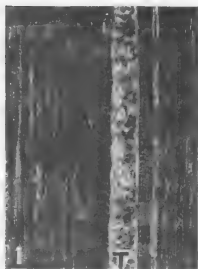


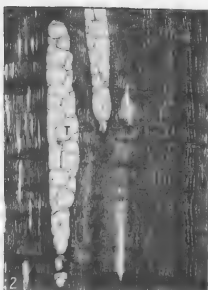
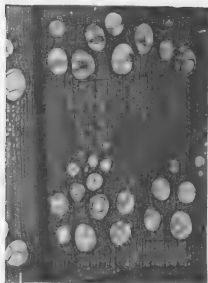
## PLATE LII

Fig. 1.—Split radial face of a creosoted hickory block, showing tyloses (*T*) in a large vessel. Magnified 12 diameters. Tyloses uncolored; remaining wood substance black with creosote.

Fig. 2.—Tangential section of *Aesculus octandra*, yellow buckeye  $\times 680$ , showing two tyloses (*T*) which have grown out of one medullary-ray parenchyma cell (*MR*). Shows open connection between the tyloses and parenchyma cell.

Fig. 3.—Cross section of valley oak, a white oak, showing young tyloses (*T*) next the bark (*B*) in vessels (*V*).





### PLATE LIII

Fig. 1.—Cross section of a white oak, showing fully developed tyloses (*T*) in the large vessels (*V*).

Fig. 2.—Radial-longitudinal view, quarter-sawed surface, of the white oak shown in figure 1, showing complete closing of the vessel (*V*), which makes this wood valuable in light cooperage, etc.

Fig. 3.—Cross section of sapwood of pignut hickory, showing fully developed tyloses (*T*).

Fig. 4.—Radial view of mesquite, showing "gum" droplets (*G*) and formations often stimulating tyloses.

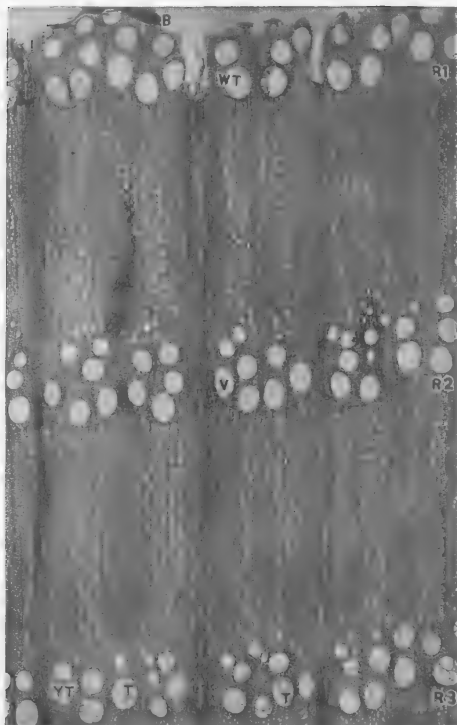
PLATE LIV

Cross section of cow oak, a white oak, showing normal and abnormal tyloses. From top to bottom are bark (*B*) and three annual growth rings (*R*<sub>1</sub>, *R*<sub>2</sub>, *R*<sub>3</sub>).

Fig. 1.—Wound tyloses (*WT*) induced by the felling of the tree and the sudden cessation of sap flow.

Fig. 2.—No tyloses (*V*); empty vessels. Normal tyloses not yet developed.

Fig. 3.—Young (*YT*) and well-developed normal tyloses (*T*).



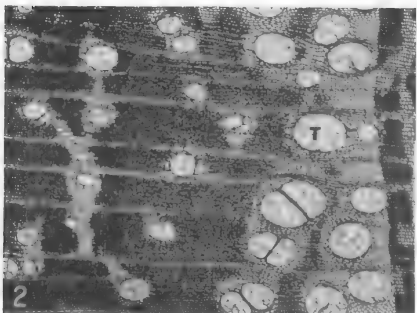
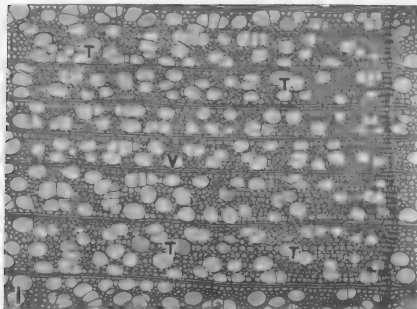


PLATE LV

Fig. 1.—Cross section of a diffuse porous wood, yellow poplar or tulip, showing scattered tyloses  $\times 50$ . *T*, tylose-filled vessels; *V*, empty vessels.

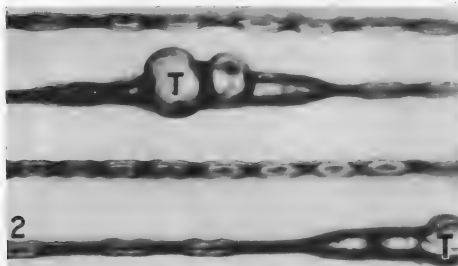
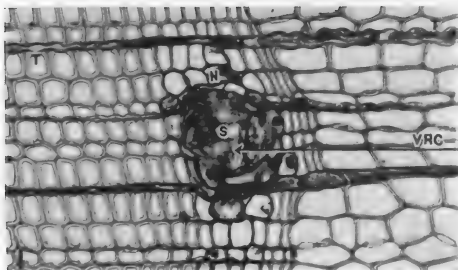
Fig. 2.—Cross section of a ring porous wood, osage orange, with vasicentric parenchyma, showing abundantly developed tyloses (*T*)  $\times 50$ .



PLATE LVI

Fig. 1.—Cross section of western white pine, showing ray tyloses (*T*), closed vertical resin canal (*VRC*) in young sapwood, and nuclei (*N*) visible in epithelial cells of canal which is beginning to split open at *S*.

Fig. 2.—Tangential section of Norway pine, showing ray tyloses (*T*).



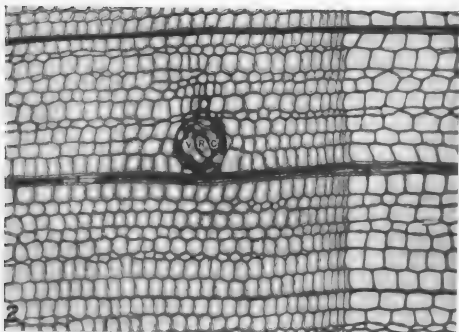
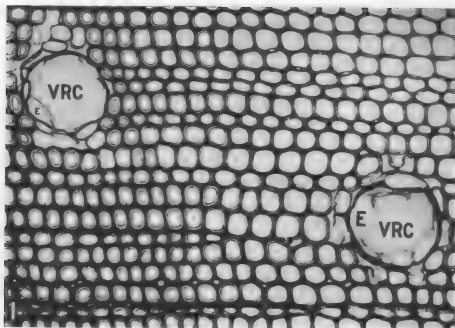


PLATE LVII

Fig. 1.—Cross-section view of shortleaf pine, showing open and partly closed vertical resin canals (*VRC*). These are typical of many canals in pine heartwood. Shows thin-walled epithelial cells (*E*).

Fig. 2.—Heartwood of Sitka spruce, showing closed vertical canal (*VRC*).

## PLATE LVIII

Open and closed horizontal canals in sapwood.

Fig. 1.—Open canal in tamarack (*TE*) thick-walled epithelium.

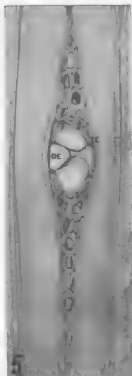
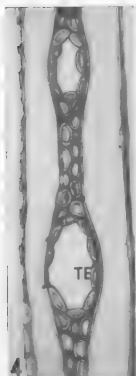
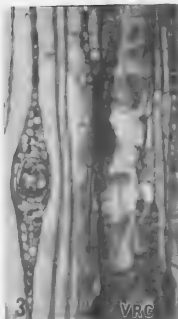
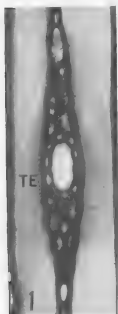
Fig. 2.—Partly closed canal with distended epithelial cells (*DE*) in Douglas fir.

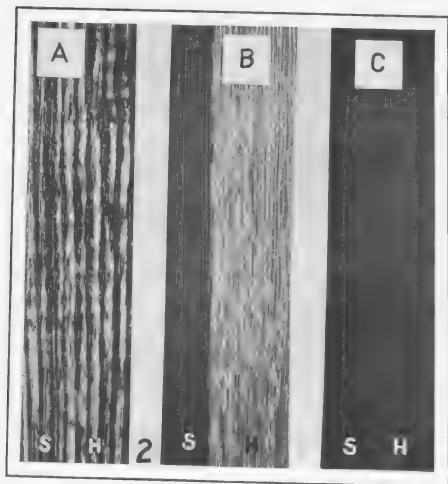
Fig. 3.—Young canal which has never opened in western white pine. Cells with protoplasm and nuclei. Vertical canal (*VRC*) in same condition on right; this is longitudinal view of same canal as is shown in cross section, Plate LVI, figure 1.

Fig. 4.—Open canal in red spruce surrounded by thick-walled epithelium (*TE*).

Fig. 5.—Partly closed canal in red spruce. *TE*, thick-walled, and *DE*, thin-walled distended epithelial cells.

Fig. 6.—Closed canal in Engelmann spruce. From old sapwood. The epithelial cell has completely closed the canal and its wall has become thickened.





## PLATE LIX

Fig. 1.—Log from collection of woods in the Forest-Products Laboratory—a specimen of the material used in this study; *S*, sapwood; *H*, heartwood.

Fig. 2.—Specimens of woods showing creosote penetrance in sap and heartwood as affected by tyloses. The three specimens each contain both sapwood and heartwood. Specimen *A*.—Red oak. Has no tyloses; creosote passed chiefly down the large vessels; note black streaks. Wood substance between vessels little treated; note white streaks. Specimen *B*.—White oak. Has abundant tyloses in sap and heartwood. Creosote penetrated the sapwood only. Thorough absorption obtained in the sapwood substance between the impenetrable, tylose-filled vessels. Specimen *C*.—Pignut hickory. Has abundant tyloses in sap and heartwood. Creosote penetrated both. Good absorption throughout in the wood substance between the tylose-filled vessels. Compare Plate LII, figure 1, an enlarged view of a portion of this block.



## THE CAMBIUM MINER IN RIVER BIRCH

By CHARLES T. GREENE,

*Entomological Assistant, Forest-Insect Investigations,  
Bureau of Entomology*

The species of the family Agromyzidæ generally mine in the leaves and stems of various plants, while some mine in their roots. The species presented in this paper, *Agromyza pruinosa* Coq.,<sup>1</sup> is quite out of the ordinary in that it mines in the cambium of the living tree, the mine leaving a scar known as a "pith-ray fleck."<sup>2</sup> These flecks in the various kinds of wood have been known for many years to be the result of the work of insects, and extensive investigations have been carried on in Europe as well as in this country in order to determine the species causing the damage. Investigations in Europe have proved that at least the pith-ray fleck in birch may be accredited to *Agromyza carbonaria*,<sup>3</sup> which is closely related to the American species. The pith-ray flecks in birch in America have been studied carefully, and it has been decided that *Agromyza pruinosa* is at least one of the insects that produce flecks and is possibly the only one. *Agromyza pruinosa* taken from river birch has just been reared to maturity. This is the first record in America of the production of flecks in birch by a definitely known species. (Pl. LX, fig. 2.)

### SEASONAL HISTORY

During July and the early part of August, 1912, the work of this dipterous larva was very common in river birch at the Chain Bridge, in the District of Columbia, every tree that was examined containing new work; but in 1913, in the same locality, only a few trees disclosed new work. A dipterous larva and similar work were found frequently in red maple (*Acer rubrum*), but not so commonly as in birch. In 1913 Mr. T. E. Snyder found in wild cherry (*Prunus* sp.) on the Virginia shore of the Potomac River at the Chain Bridge two larvæ which are identical with the larvæ of *Agromyza pruinosa* in the birch, except that they are only two-thirds as long, although to all appearances full grown. The work of this species in wild cherry is identical with that in red maple and black birch, but the mines are correspondingly smaller.

<sup>1</sup> Thanks are due to Mr. J. R. Malloch for assistance in determining the species.

<sup>2</sup> Brown, H. P. Pith-ray flecks in wood. U. S. Dept. Agr., Forest Serv., Circ. 215, 15 p., 6 pl. May 7, 1913.

<sup>3</sup> Nielsen, J. C. Zoologische Studien über die Markflecke. Zool. Jahrb., Abt. System., Geogr. u. Biol. Tiere, Bd. 23, Heft 6, p. 725-738, pl. 30. 1906.

## CHARACTER OF TREES ATTACKED

The trees attacked are apparently healthy, and infested ones can not be detected by their outward appearance. The only way in which to detect the larva is to remove the bark and expose the cambium, where at a glance you can generally recognize the new galleries from the old ones, since new larval mines are only faintly darker than the living cambium; in fact, they are sometimes of a delicate pink color, whereas all the old work is generally dark brown. In Vilas and Oneida Counties, Wis., the trees in the vicinity of Tomahawk and Trout Lakes were carefully examined by Mr. S. A. Rohwer last fall (1913), and no evidence of the cambium miner was found in white birch (*Betula populifolia*), red oak (*Quercus rubra*), red maple (*Acer rubrum*), or sugar maple (*Acer saccharum*).

Pith-ray flecks were found in red oak (*Quercus rubra*) at Charter Oak, Pa., by Mr. T. E. Snyder and in mountain holly (*Ilex monticola*) at Endeavor, Pa., by Mr. F. C. Craighead, but the particular insect or insects causing them are not yet known.

## LIFE HISTORY OF THE SPECIES

## METHODS OF REARING

Numerous experiments were conducted while rearing this species. All the breeding jars were placed in a pasteboard box, which was put in an ordinary soap box lined and covered with about five thicknesses of newspaper. This box was kept outside during the winter in an inclosed shed. The frost penetrated all the protective coverings, but not so thoroughly as though the boxes had been completely exposed. Jars containing earth and sand gave the best results in these rearing experiments. From April 15 to May 12, 1913, six adults emerged. On May 1 a single adult which was reared from the larva emerged, a hymenopterous parasite emerging from another pupa case on May 13.

## THE EGG

The writer unfortunately did not succeed in securing the egg of this species, but it is apparently deposited in the fork of two branches which are about 5 to 8 years old and near the top of the tree. From the shape of the ovipositor (Pl. LXI, fig. 4) the egg is more than likely deposited on the outside of the bark, as the mine, which has been traced from a twig to the base of the tree, a distance of 40 feet, starts from this point like a hair line and, increasing in width as it goes down the trunk, reaches a width of one-eighth of an inch at the base.

THE LARVA<sup>1</sup>

The larva (Pl. LXI, fig. 1) is white, opaque, and cylindrical, averaging from 20 to 25 mm. in length and 1 mm. in diameter. One larva, collected

<sup>1</sup> The larva of this species was discovered by Mr. H. P. Brown and was first shown to the writer by Mr. T. E. Snyder.

on June 19, 1913, was 30 mm. in length and 1 mm. in diameter. The hooklet is shiny black and chitinized, the exposed portion being more highly chitinized than the rest. The hooklet complete (cephalopharyngeal skeleton) dissected out is shown in Plate LXI, figure 1, *a*. Back of the large hooklet are two smaller toothlike processes, one on each side, the position of these being shown at *b*. The anterior spiracles at *c* and the posterior pair at *d* are a very pale yellow, and their position is shown in outline. At the caudal end of the larva are two padlike surfaces, very faintly raised from the surface of the body, reaching nearly around the circumference of the body and covered with numerous brown, hooklike hairs or bristles. Several stages of the larvæ were observed, and the only noticeable difference was in their size.

If the larva reaches the base of the tree before the time to pupate, it will turn and mine up the cambium for some distance; on one occasion the larva retreated for 6 feet, then returned, thus encircling the root, and followed it for 2 feet from the trunk. The exit hole is sometimes made on the side of the root, but generally it is on the underside, and the larva pupates immediately on emergence. The pupæ were found from one-half to one inch from the exit hole. A portion of river birch (*Betula nigra*) with the bark removed is shown in Plate LX, figure 1, to illustrate the larval mines, while figure 2 is part of a cross section showing the "pith-ray flecks" from above.

The only larva that was reared by the writer, and in fact the only one that reached maturity, was placed in a large vial July 30, 1912, with a piece of freshly cut river-birch bark, the inner surface of which was covered freely with fresh sap. A piece of gauze was placed over the opening of the vial. On August 6, 1912, at 8.30 a. m., the larva commenced pupation, first becoming rigid and then changing to deep yellow at both ends, while the central portion remained the natural white color. It was 25 mm. in length and 1 mm. in diameter, but by noon it had decreased to about 10 mm. in length and increased to 2 mm. in diameter. Both ends had changed to dark brown and were perfectly formed, as in the pupa, and the middle was a light yellowish. At 5 p. m. the pupa was perfectly formed and dark brown all over, its dimensions now being 5 mm. in length and 2 mm. in diameter. The larva pupated under the thin folds of the outer bark, as there was nothing else in the vial.

#### THE PUPA <sup>1</sup>

The pupa (Pl. LXI, fig. 2) is of the usual cylindrical type and dark reddish brown in color, averaging from 4 to 5 mm. in length by 2 mm. in diameter, and is formed by the shrinking of the larval skin. The anterior spiracles are slightly more prominent than the posterior pair.

---

<sup>1</sup>The pupa of the species was discovered and first shown to the writer by Mr. T. E. Snyder.

## THE ADULT

The adult (Pl. LXI, figs. 3 and 4) of *Agromyza pruinosa* Coq.,<sup>1</sup> six specimens of which were reared by the writer in the spring of 1913, is closely related to *Agromyza carbonaria* Zett. of Europe. *Agromyza pruinosa* remains in the pupal stage in the ground during the winter and emerges from the pupa case in one of two ways: Either the end of the pupal case is pushed off completely, or emergence is accomplished by tearing the end of the pupal case into shreds. Of the six specimens just referred to five were males and one a female. This species of *Agromyza* is represented in the United States National Museum collection by Coquillett's type, a single male specimen (Catalogue No. 6659, U. S. National Museum). The writer's specimens agree perfectly with the type, except that they are very slightly larger.

The general appearance of the adult female corresponds to that of the male, with the exception that it is slightly more robust. The ovipositor is slightly over one-half of a millimeter in length, chitinized, and somewhat shiny on the sides and edges of the dorsal surface. It is slightly flattened and a little broader at the apex than at the base. On the dorsal surface is a granular space, rounded toward the base of the ovipositor.

The total length of the female is 4 mm., and of the male about 3 mm. The abdomen of the female is shown in figure 4 of Plate LXI.

In an adult that had just emerged from the pupal case, the eyes were brownish and the frons and face a pale yellow or orange color. The thorax was pale gray, the legs yellowish, and the wings opaque white, clearing to hyaline in about two hours. The abdomen was of a dull orange color, with a faint gray line along the edge of each segment. The whole insect assumed its natural color in two and a half hours.

## A HYMENOPTEROUS PARASITE

On May 13, 1913, a hymenopterous parasite, *Sympha agromyzae* Rohwer<sup>2</sup> (Pl. LXI, fig. 5), issued from a pupa case of *Agromyza pruinosa* Coq. This parasite is nearly as large as its host. Apparently it deposits its egg within the egg of the host. The apparently normal dipterous larva mines down the tree trunk and enters the ground; the pupa is perfectly formed, outwardly exhibiting no signs of parasitism, but about the time the host should emerge the parasite issues instead. At maturity the end of the pupal case is pushed open by the parasite in the same manner as the host would do it.

<sup>1</sup>Coquillett, D. W. New acalyprate Diptera from North America. Jour. N. Y. Ent. Soc., v. 10, No. 4, p. 177-191. Dec., 1902. "*Agromyza pruinosa*, sp. nov.," p. 189.

<sup>2</sup>"*Sympha agromyzae*, n. sp. Female. Length 3 mm. Notauli well defined; prescutum with a foveolate furrow; face sparsely punctured; propodeum with a transverse carina; hind tarsi pale. Type Cat. No. 16474 U. S. Nat. Mus." (S. A. Rohwer). A detailed description will appear later in the Entomological News.

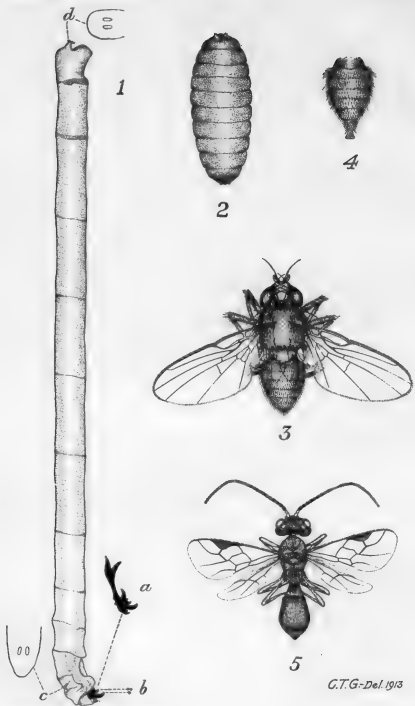
PLATE LX

Fig. 1.—River birch with bark removed, showing larval mines of *Agromyza pruinosa*.

Fig. 2.—Section through wood of river birch, showing "pith-ray flecks" produced by the work of *Agromyza pruinosa*.

Photographed by H. B. Kirk.





G.T.G.-Del. 1913

PLATE LXI

- Fig. 1.—*Agromyza pruinosa*: Larva and details.  
Fig. 2.—*Agromyza pruinosa*: Pupa.  
Fig. 3.—*Agromyza pruinosa*: Adult male.  
Fig. 4.—*Agromyza pruinosa*: Abdomen of adult female, showing ovipositor.  
Fig. 5.—*Sympha agromyzae*: Adult.



# A STUDY OF SOME IMPERFECT FUNGI ISOLATED FROM WHEAT, OAT, AND BARLEY PLANTS

BY EDWARD C. JOHNSON,

*Formerly Pathologist in Charge of Cereal-Disease Investigations,  
Bureau of Plant Industry*

## INTRODUCTION

Of the imperfect fungi, many are parasitic on cereals wherever climatic conditions favor their development. They occur as scab on the heads, as leaf spots, and as infections in the culms and roots. Usually one or more species are present in the roots and culms of stunted plants, more particularly where some one cereal crop has been grown year after year on the same land. A study of the fungi occurring on wheat, oats, and barley, with particular reference to their pathogenicity, is therefore of much economic importance.

Such a study was begun in the cereal-disease laboratory of the Office of Grain Investigations of the Department of Agriculture in 1910. Species of imperfect fungi were isolated from wheat, oats, and barley obtained from various parts of the country. *Helminthosporiums*, *Alternarias*, *Cladosporiums*, and *Fusariums* were obtained. They were secured from leaf spots or from the lower nodes, root crowns, or roots of more or less stunted plants. In many cases they were obtained pure from fresh sporulating material on leaves and stems. In other cases they were obtained from the nodes, root crowns, and roots by sterilizing these parts externally in a 1 to 1,000 solution of mercuric chlorid, washing them in several changes of sterile water, and incubating them in moist chambers. After incubation for three to five days at a temperature of 72° to 77° F., sporulating myceliums were usually obtained. Plate cultures were then made and the fungi present isolated in pure cultures and propagated. On corn-meal agar, corn meal, and potato cylinders most of them grew and sporulated profusely.

Pure cultures were obtained and grown and the identity determined as *Fusarium culmorum* W. G. Sm., *Helminthosporium gramineum* Rabh., *Cladosporium gramineum* Cda., and a species of *Alternaria*. The determination of *Fusarium culmorum* was made by Dr. H. W. Wollenweber, of the Bureau of Plant Industry; the other determinations were made by the writer. *Helminthosporium gramineum* was isolated from the lower parts of the culms of stunted wheat plants growing on land continuously cropped to wheat at the Minnesota Agricultural Experiment Station, and from wheat leaves and barley leaves at the same

place. *Cladosporium gramineum* was obtained from the leaves of oats at the same station. The *Alternaria* species occurring with *Helminthosporium* or independently were isolated from wheat culms in the same manner as *Helminthosporium gramineum*. *Fusarium culmorum* was isolated from wilted oat plants obtained from a 10-acre field on the farm of Mr. Peter Hanson, Sandy, Utah, on May 10, 1910. On this farm about 10 acres of oats had been practically destroyed by disease a few weeks after the seed was planted. The plants sent to the cereal-disease laboratory for examination and diagnosis were sterilized by immersing them in a 1 to 1,000 mercuric-chlorid solution for 10 minutes, followed by washing in sterile water. They were then placed in a moist chamber at a temperature of about 75° F. for several days and were soon covered with a luxuriant fungous growth. This proved to be a pure culture of *Fusarium culmorum*. It was plated and grown on potato cylinders and corn meal and sporulated abundantly.

After securing these fungi in pure cultures and inducing profuse sporulation, tests were made as to their pathogenicity on the leaves, seeds, and seedlings of wheat, oats, barley, and rye.

#### INOCULATION OF LEAVES OF WHEAT, OATS, BARLEY, AND RYE WITH SPECIES OF IMPERFECT FUNGI

Seedling plants of wheat (Haynes Bluestem, Minn. No. 169), oats (Early Gothland, Minn. No. 26), barley (Manchuria, Minn. No. 105), and rye (winter) were grown in the greenhouse at Washington, D. C., in 6-inch pots under temperature and moisture conditions as nearly normal as possible. When the seedlings were 2 to 3 inches high, inoculations were made about an inch from the leaf tip, with spores transferred from pure cultures in test tubes by means of a flattened inoculating needle, care being taken that little or none of the nutrient medium was transferred to the leaves. If any of the medium accompanied the spores, control plants were similarly treated with the same medium minus the spores. Care was taken not to injure the leaves in any way. The inoculated plants were placed under bell jars standing in pans of sand and water, thus permitting the moisture transpired to condense on the leaves, making an ideal condition for spore germination. They were allowed to remain under the bell jars for 48 hours and were then removed and placed in the greenhouse at a temperature ranging from 55° to 65° F. Table I shows the results of these inoculations.

TABLE I.—Results of inoculating seedling leaves of wheat, barley, oats, and rye with imperfect fungi obtained from cereals.

Test No.	Species.	Origin.	Inoculated on—	Date of inoculation.	Length of incubation.	Number of inoculations.	Infection.		Control.	
							Num-ber.	Per-cent-ge.	Total num-ber.	Num-ber in-fected.
1	<i>Helminthosporium gramineum</i> .	Wheat node <sup>1</sup>	Wheat.	1911. Oct. 31	Days. 6	21	21	100	11	1
2	do.	do.	do.	Nov. 10	4	33	17	51	12	0
3	do.	do.	Barley.	Oct. 31	6	26	23	84	12	0
4	do.	do.	do.	Nov. 10	4	50	39	78	12	0
5	do.	do.	Oats.	Oct. 31	6	24	16	66	7	0
6	do.	do.	do.	Nov. 10	4	25	6	24	12	0
7	do.	do.	Rye.	do.	4	36	8	22	14	0
8	do.	Barley leaf <sup>1</sup>	Wheat.	1912. Jan. 24	5	43	40	93	15	0
9	do.	do.	Barley.	do.	5	67	67	100	25	0
10	do.	do.	Oats.	do.	5	50	50	100	20	0
11	do.	do.	Rye.	do.	5	45	45	100	22	0
12	<i>Cladosporium gramineum</i> .	Oat leaf <sup>1</sup>	Wheat.	do.	5	35	0			
13	do.	do.	do.	do.	6	47	0			
14	do.	do.	Barley.	do.	5	44	0			
15	do.	do.	do.	do.	6	64	0			
16	do.	do.	Oats.	do.	5	37	0			
17	do.	do.	do.	do.	6	108	0			
18	do.	do.	Rye.	do.	5	37	0			
19	do.	do.	do.	do.	6	32	0			
20	<i>Fusarium culmorum</i> .	Oat seedling <sup>2</sup>	Wheat.	Mar. 19	14	50	0			
21	do.	do.	Barley.	do.	14	50	0			
22	do.	do.	Oats.	do.	14	50	0			
23	do.	do.	Rye.	do.	14	50	0			

<sup>1</sup> From University Farm, St. Paul, Minn.<sup>2</sup> From farm of Mr. Peter Hanson, Sandy, Utah.

Table I shows that the strains of *Helminthosporium gramineum* from both wheat and barley infected the leaves of wheat, barley, oats, and rye. On wheat, barley, and rye the leaf spots at the point of inoculation became distinct in a little less than three days after inoculation. These spots, which had a dead central area surrounded by a brown margin, slowly increased in size until their diameter was almost equal to the width of the leaf. A tendency to striation of the leaf area contiguous to the spots was noticed. No striking difference could be detected in the effect of the fungus from wheat or barley, the strain from wheat attacking barley and rye fully as severely as the strain from barley, and the strain from barley attacking wheat and rye fully as severely as the strain from wheat. On oats the two strains showed a slight difference in virulence, the fungus isolated from the barley apparently showing greater vigor in its attack than the fungus from wheat. In fact, three days after inoculation with the fungus from barley, oat leaves were so severely affected that in many cases they were cut in two, the tip portion often breaking off and falling to the ground. The two strains behaved so similarly, however, that physiologically they undoubtedly may be regarded as identical. Morphologically, no difference was detected.

Table I also shows that *Cladosporium gramineum* and *Fusarium culmorum* did not form leaf spots, even though the number of inoculated leaves was fairly large. This was rather unexpected in the case of *Cladosporium*, as it was obtained in pure culture by plating direct from a fresh mass of spores from a badly infected oat leaf in the field. Continuous culture on artificial media apparently either reduced its virulence, the temperature and moisture conditions in the greenhouse not being such as were conducive to infection by this fungus, or infection took place normally only after aphid injury or other wound. That *Fusarium culmorum* did not produce leaf spot was to be expected, as it usually does not occur in this manner and was not isolated from a leaf but from a wilted plant.

#### INOCULATION OF SEED OF WHEAT, OATS, BARLEY, AND RYE WITH SPECIES OF IMPERFECT FUNGI

Seed of wheat, oats, barley, and rye was inoculated with spores of the same strains of imperfect fungi used in the seedling-leaf inoculation tests. The fungi were grown in pure cultures in the same manner as those used for the leaf inoculation work. When sporulating profusely, sterile water was poured into the test tubes, the spore masses were loosened by the use of platinum needles, and the contents were well shaken. The water containing the spores was then poured off and diluted with sterile water until a drop placed under the microscope was found to contain from 5 to 25 or more spores. Seed of wheat, barley, oats, and rye was sterilized by immersion for one hour in a formalin solution consisting of 2.5 parts of 40 per cent formaldehyde to 1,000 parts of water and was immediately dried and inoculated with spores by soaking it in the water containing them. The seed was then planted in 6-inch pots filled with a sandy loam soil rich in humus and placed in the greenhouse at temperatures ranging from 55° to 65° F. The soil used had been sterilized previously in a steam sterilizer at a pressure of 15 pounds for two hours, the temperature being approximately 265° F. Control seed which had been sterilized but not inoculated was planted for comparison in every case. The results from such inoculation and plantings in the greenhouse are shown in Table II.

TABLE II.—Results of inoculating seed of wheat, barley, and oats with imperfect fungi isolated from grain plants.

Test No.	Species.	Origin.	Inoculated on—	Date of planting.	Inoculated seed.			Control seed.		
					Number planted.	Germinated.		Number planted.	Germinated.	
						Number.	Percentage.		Number.	Percentage.
1	<i>Helminthosporium gramineum</i> .	Wheat culm....	Wheat.	1911. Nov. 21	150	34	22.6	90	68	75.5
2	do	do	do	Dec. 2	72	25	34.8	112	93	83.0
3	do	do	do	do	78	18	23.0	78	51	65.3
4	do	do	do	1912. Jan. 19	105	64	60.9	105	92	87.6
5	do	do	do	1911. Dec. 2	112	30	26.7	112	93	83.0
6	do	do	Barley.	do	105	87	82.8	105	83	79.0
7	do	do	Oats.	do	70	55	78.5	70	57	81.4
8	do	Barley leaf.	Wheat.	Nov. 19	105	62	59.0	105	92	87.6
9	do	do	Barley.	do	105	86	81.9	105	83	79.0
10	do	do	Oats.	do	70	52	74.2	70	57	81.4
11	<i>Fusarium culmorum</i> .	Oat seedling....	Wheat.	1912. Mar. 1	96	22	22.9	96	80	83.3
12	do	do	Barley.	do	96	63	65.5	96	85	88.5
13	do	do	Oats.	do	120	2	1.7	80	68	85.0
14	<i>Alternaria</i> sp.	Wheat culm....	Wheat.	1911. Nov. 21	150	108	61.2	90	73	81.1
15	do	do	do	Dec. 2	112	92	82.0	112	93	83.0
16	do	Wheat seedling.	do	1912. Mar. 5	80	79	98.7	80	75	93.7
17	do	do	Barley.	do	80	69	86.2	72	62	86.1
18	do	do	Oats.	do	90	77	85.5	90	79	87.7
19	<i>Cladosporium gramineum</i> .	Oat leaf.	Wheat.	Feb. 28	112	105	93.7	84	78	92.8
20	do	do	Barley.	do	110	96	87.2	84	70	83.3
21	do	do	Oats.	do	112	104	92.8	84	72	85.7

Table II shows that the strains of *Helminthosporium gramineum* isolated from wheat and barley were decidedly pathogenic to germinating wheat, only 22 to 60 per cent of the inoculated wheat in five trials producing plants, while 65 to 87 per cent of the controls not inoculated produced sound plants. These results are shown further in Plate LXII, figure 1. Barley and oats were not affected to any appreciable degree so far as germination and sprouting were concerned, the inoculated seed producing as large a percentage of plants as the clean seed. Those wheat plants which developed from inoculated seed were stunted and not nearly so vigorous as those produced from clean seed. At the end of six weeks the difference in height of plants from inoculated and clean seed was very marked. The plants from seed inoculated with *H. gramineum* from wheat were 5.5 inches high to the tip of the second leaf and those from seed inoculated with *H. gramineum* from barley 4.88 inches high to the tip of the second leaf, while control plants grown from clean seed averaged 6.45 inches high to the tip of the second leaf.

A similar difference was noticeable in barley plants grown from inoculated and clean seed, although the difference was not quite as marked

as in the wheat plants. This is shown in Plate LXII, figure 2. Barley plants from seed inoculated with *Helminthosporium gramineum* from barley were 5.82 inches high at the end of six weeks, those from seed inoculated with *H. gramineum* from wheat were 6.34 inches high, and those from clean seed, 6.46 inches high. The measurements are the averages of 50 plants in each case. There was no measurable difference in the height of oat plants grown from inoculated and from clean seed. *H. gramineum* was easily reisolated in every trial both from stunted wheat and stunted barley plants by external sterilization in mercuric-chlorid solution and incubation at room temperature.

*Fusarium culmorum* was even more virulent than *Helminthosporium gramineum*, particularly on oats. Inoculated wheat seed produced only 22.9 per cent of sound plants, barley seed 65.5 per cent, and oat seed only 1.7 per cent, while the controls produced 83.3, 88.5, and 85 per cent of sound plants, respectively. The results of the inoculations are further strikingly shown in Pl. LXII, figures 3, 4, and 5. The 10-acre oat field where this fungus was secured had been practically destroyed by some disease, and these results show that *F. culmorum* undoubtedly was the causal organism.

The two strains of *Alternaria* sp., one isolated from wheat culms from University Farm, St. Paul, Minn., the other from wilted wheat seedlings from Vermont, had no pathogenic effect on wheat, oats, or barley, the differences in percentage of germination from inoculated seed and control seed being so slight as to be negligible. *Cladosporium gramineum* also had very little if any effect on the seedlings, the percentage of germination from inoculated seed being only slightly smaller than from control seed.

To determine further how the *Helminthosporium gramineum* attacked the seed and seedlings, a large number of seeds and seedling plants grown from inoculated seed were dug and examined a few days after germination. It was found that many of the seeds had been attacked by the fungus so rapidly that they had not had an opportunity to germinate. Many others had germinated, apparently became infected immediately, and were killed before they were an inch high. Plants which survived were severely affected, as shown by the brown discoloration at the base of the culms, a condition not noticed in any of the controls. This discoloration usually occurred in the basal leaf sheath. When the plants had grown for several weeks, it was also very noticeable in the root crown. The discoloration was not as marked in barley grown from inoculated seed as the discoloration in wheat and was entirely absent in oats.

Numerous seeds and seedlings inoculated with *Fusarium culmorum* were also examined. Many seeds were found to have been killed before the process of germination had proceeded sufficiently far for any roots to form and before the plumule emerged from the ground. Eight days after

planting, the whole seed often was permeated by the fungus, the contents of these coats having a pink coloration. The plants which survived were discolored at the base in a manner similar to those of plants from seed inoculated with *Helminthosporium gramineum*. Where discolorations occurred, it was the first leaf sheath which was affected, while the central stem or culm was normal in appearance and color. The vigor of the plants from inoculated seed was markedly reduced, and they were shorter than the normal plants during the six weeks in which they were grown. This was true also of wheat and barley grown from seed inoculated with this fungus.

#### COMPARATIVE ROOT DEVELOPMENT OF WHEAT PLANTS GROWN FROM SEED INOCULATED WITH *HELMINTHOSPORIUM GRAMINEUM* AND FROM CLEAN SEED

To determine the comparative development of the root systems of surviving plants from seed inoculated with *Helminthosporium gramineum* and from clean seed, two pots of wheat containing five plants each, one grown from inoculated and the other from clean seed, were removed to the laboratory and the soil carefully washed away from the root systems. The roots were spread out by floating them in water and then drawing off the water. The difference in development of the root systems of the two sets of plants was very marked. The roots of plants from inoculated seed were discolored near the root crown. They were also much shorter and much less vigorous than roots of plants from clean seed; this is strikingly shown in Plate LXIII. Numerous other plants were examined, and it was found that in practically every case where inoculated seed had produced plants which survived, the root systems were less vigorous than in plants grown from clean seed.

#### SOIL INFECTION WITH *HELMINTHOSPORIUM*

To determine whether or not soil in which seed inoculated with *Helminthosporium gramineum* had been planted would remain sufficiently infected for any length of time to injure later plantings, inoculated seed was planted in pots in the greenhouse at Washington, D. C., on November 21, 1911, and the resulting plants were grown for five weeks and then cut off. Control pots were similarly planted with clean seed and the plants removed after five weeks. These pots were again sown on January 13, 1912, with wheat which had been previously sterilized in a 2.5 to 1,000 formalin solution. Of 150 seeds planted in the soil in which wheat plants had been grown from seed inoculated with *H. gramineum*, 104, or 69.3 per cent, germinated and produced plants, while of 90 seeds planted in control pots 76, or 84.4 per cent, germinated. This indicates that the soil remained infected during the two months in which the experiment was in progress. How long soil remains infected in this way is one of the important problems in plant pathology.

## FIELD EXPERIMENTS WITH SEED INOCULATED WITH IMPERFECT FUNGI

In order to test whether the imperfect fungi which were found pathogenic in the greenhouse on seeds and seedlings would act similarly under field conditions, field experiments were undertaken at University Farm, St. Paul, Minn., in the spring of 1912.<sup>1</sup> The two strains of *Helminthosporium gramineum* and the one strain of *Fusarium culmorum* which had been found pathogenic in the greenhouse were tested in connection with wheat, barley, and oat seed. The same varieties of grains which were used in the experiments at Washington, D. C., were used in the field experiments. The seed was treated in a formalin solution of 3 parts of 40 per cent formaldehyde to 1,000 parts of water for one hour and afterwards was inoculated exactly as in the greenhouse work already described. Immediately after inoculation, the seed was planted in the field in rows 1 rod in length and 10 inches apart, with controls every alternate two rows. The seeds were counted. After the grain had sprouted and the plants were from 3 to 6 inches high, careful counts were made to determine the percentage of germination and observation made of the vigor of the plants during the first few weeks of growth. The results are given in Table III.

TABLE III.—Results of inoculating seed of wheat, barley, and oats with imperfect fungi isolated from grain plants, and of planting them in the field at University Farm, St. Paul, Minn.

Test No.	Species.	Origin.	Inoculated on—	Date of planting.	Inoculated seed.			Control seed.		
					Number planted.	Germinated.		Number planted.	Germinated.	
						Number.	Percentage.		Number.	Percentage.
1	<i>Helminthosporium gramineum</i> .	Wheat culm...	Wheat...	1912. Apr. 27	160	125	78.1	159	155	97.5
2	do.	do.	Barley	do.	160	115	71.9	160	130	81.2
3	do.	do.	Oats	do.	160	145	90.6	160	142	88.7
4	do.	Barley leaf.	Wheat	do.	160	89	55.6	160	122	76.2
5	do.	do.	Barley	do.	160	109	61.8	160	131	81.9
6	do.	do.	Oats	do.	160	121	75.6	160	142	88.7
7	<i>Fusarium culmorum</i> .	Oat seedling..	Wheat	do.	160	98	61.2	160	130	81.2
8	do.	do.	Barley	do.	160	114	71.2	160	130	81.2
9	do.	do.	Oats	do.	160	96	60.0	160	140	87.5

The results given in Table III substantiate the results of the experiments in the greenhouse. *Helminthosporium gramineum* from wheat when applied to the seed reduced the percentage of germination of both wheat and barley, but not to the same extent as in the greenhouse tests. Oats were not appreciably affected. The material used for inoculation

<sup>1</sup> In these experiments the writer was assisted by Messrs. Alden A. Potter and John H. Parker.



was not in a profusely sporulating stage and therefore not in as active a condition as the material which was used in the inoculations in the greenhouse. The seed which was inoculated also was still slightly damp after the treatment in the formalin solution and this trace of formalin might have reduced the effectiveness of the spores to some extent. The strain of *H. gramineum* from barley was more virulent than the strain from wheat, the percentage of germination being less where this strain was used for inoculation than where the strain from wheat was used. After inoculating with this strain, even the germination of the oats was considerably affected. The material used for inoculation, however, was in better condition than the material of the strain from wheat, as the fungus was sporulating abundantly when used. The plants of both wheat and oats which survived were less vigorous than the plants from clean seed, being slightly smaller than the plants in the control rows.

*Fusarium culmorum* also was virulent, particularly on oats, and its effect on wheat and barley was marked. The wheat plants which survived after inoculation with this fungus were smaller than those in the control rows, the difference being measurable. Several of the plants were dying when counted. In the case of barley the difference in the plants from inoculated seed and control seed was not marked, while in the case of oats many plants from the inoculated seed were very weak when counted, the difference in vigor between them and plants from clean seed being very noticeable. There was a sufficient difference in stand between rows from inoculated and from clean seed in the case of wheat, oats, and barley to be noticeable even without counting the plants.

That the reduction in germination and injury to seedlings was less marked in the field experiments than in the greenhouse experiments may be due to several causes. The temperatures in the field were considerably lower than under greenhouse conditions, and the fungi may have been less active for that reason. Again, the grain which had been treated with a formalin solution was not absolutely dry when inoculated and the trace of formalin present may have reduced the vitality of the spores. One other fact, however, which may have had a marked influence is that in the field the fungi used for inoculation would have to compete with other fungi and bacteria in the soil and many of the spores may have been injured before they could germinate and infect the grain. That such competition between fungi and bacteria in the soil may not be uncommon was indicated in a preliminary experiment in the greenhouse where wheat inoculated with *Helminthosporium gramineum* was planted in sterilized and unsterilized soil. It was found that the wheat planted in the sterilized soil was more severely injured by the fungus than the wheat planted in unsterilized soil, the percentage of germination being less in the sterilized soil than in the soil not sterilized. In a second experiment of this

nature the results were not as marked as in the first, although there was a difference in germination of 3.8 per cent between inoculated wheat planted in sterilized soil and inoculated wheat planted in soil which had not been sterilized.

#### A SYNOPSIS OF WORK RELATIVE TO HELMINTHOSPORIUMS AND FUSARIUMS ON CEREALS

The most comprehensive study of Helminthosporiums on grains is that of Ravn (20)<sup>1</sup> who isolated three species from barley and oats and by cultural and inoculation experiments, as well as a study of the morphology, definitely established their identity. Eidam (12) was the first to undertake inoculation experiments with species of Helminthosporiums. He inoculated barley with a strain of Helminthosporium secured from oats, but without positive results. Ritzema Bos (21) describes some of the diseases of barley in Holland and ascribes them to *H. gramineum*. Frank (13) describes a disease of barley which appears on the lower leaves of young plants and spreads gradually upward and believes it to be due to an infection of *H. gramineum*. Ritzema Bos (22) describes a disease on oats slightly different from a leaf spot in barley and believes it to be caused by *H. gramineum*. Pammel (18) describes a characteristic barley disease appearing in the United States and believes *H. gramineum* to be the causal organism. Many other investigators, both in Europe and this country, have studied the Helminthosporiums on grains with more or less definite results, and the literature on the subject is extensive. Practically all these studies, however, have been based on examinations of diseased plants and, with the exception of the work of Eidam, already quoted, have not been based on cultural and inoculation work. Hecke (14) secured a pure culture of *H. gramineum* from barley plants. He inoculated seedling barley plants both with mycelium and sclerotia and secured positive results in the formation of brown spots on the leaves. Ravn (20) cleared up the question of identity of three species of the Helminthosporiums attacking barley and oats. In extensive cultural and inoculation studies he obtained pure cultures. One of these he secured from stunted barley plants and established that it was the cause of deep-seated infection in the tissues of leaf, stem, and roots, while another species affected only the leaves, but was not systemic. The first he attributes to *H. gramineum*, the second to *H. teres* Sac. A similar disease on oats is attributed to *H. avenae* Br. and Cav. These three fungi were studied in pure cultures on beer wort and other culture media and found to differ in cultural characteristics, *H. gramineum*, after 14 to 20 days' growth on beer wort, producing a snow-white, uniformly smooth mycelium; *H. teres*, a much less abundant mycelium,

---

<sup>1</sup> Bibliographic citations in parentheses refer to "Literature cited," pp. 487-489.

which gathers more or less in masses; and *H. avenae*, a mycelium more nearly resembling *H. gramineum*, but less smooth and with more of a tendency to mass together. The developmental history and morphology of the mycelium and conidia in culture was very similar for the three species, but when the conidia were measured in large numbers those of *H. teres* were slightly longer than those of *H. gramineum* and those of *H. avenae* slightly larger than those of *H. teres*.

In a series of inoculation experiments *Helminthosporium teres* from barley transferred to barley, but not to oats, rye, or wheat; *H. gramineum* to barley, but not to oats; and *H. avenae* to oats, very slightly to barley, and not to rye.

Until Ravn made these intensive studies of the three *Helminthosporium*s they had been confused in the literature as to identity. The strain of *H. gramineum* discussed in this paper corresponds in cultural and morphological characteristics to the descriptions by Ravn.

Pammel, King, and Bakke (19) report a number of species of *Helminthosporium* on cereals in Iowa, among them *H. gramineum*. They cite inoculation tests to show that infection occurred when barley seedlings were inoculated with spores of this fungus and when the soil in which seedlings grew was inoculated. Beckwith (5) reports the isolation of undetermined species of *Helminthosporium* from old wheat soils, roots, and stems of wheat in North Dakota, but no inoculation experiments are mentioned. A comprehensive bibliography of the literature on *Helminthosporium*s up to 1900 is given by Ravn (20).

The literature relating to *Fusarium*s on grains is also very extensive. Chester (10) reports that *F. culmorum* is the cause of the disease known as scab of wheat and shows that many shrunken wheat kernels contain a fungous mycelium. Detmers (11) shows that the disease known as wheat scab in Europe and caused by *F. culmorum* has become prevalent in America. Selby (30) ascribes wheat scab in Ohio to the fungus *F. roseum* Link. and believes the conidial form of *Gibberella saubinetti* to be its conidial stage. Some field inoculations with *Fusarium* attempted by him were unsuccessful.

The first investigator to show with any degree of certainty that *Fusarium* infection can be carried with the seed is Rostrup (25, 26, 27, 28). Ritzema Bos (24), Westerdijk (35), Volkart (34), Appel (1, 2), and Selby and Manns (31) came to similar conclusions. Sorauer (32, 33) was the first to prove that infection could be carried with the seed. He maintains, however, that infection in this manner is of small consequence as compared with infection through the soil.

Selby and Manns (31), in their studies on the form *Gibberella*, conclude that this fungus attacks rye, oats, barley, and spelt. Inoculations on wheat with pure cultures of *Gibberella saubinetti* (Mont.) Sacc. from perithecia on wheat reduced germination to the extent of 17.1 and 32.4 per cent, respectively. Similar results on both wheat and oats were obtained by them with *Fusarium roseum* from wheat and clover.

Appel (3) believed that infection with *Fusarium nivale* Ces. is due principally to soil infection, while Hiltner and Ihssen (15) believe that seed infection is of more importance.

Muth (17) carried on pure culture inoculation experiments on rye with *Fusarium roseum*. In these, 55 per cent of the inoculated seed sprouted while only 63 per cent of the controls sprouted. A large number of plants from inoculated seed, however, showed the results of infection through a yellowish or yellowish brown discoloration of the roots.

Beckwith (4) reports numerous isolations of *Fusarium* species and other imperfect fungi from stems and roots of wheat grown on soil continuously cropped to wheat and from the soil itself.

Mortensen (16) demonstrated that rye seed heavily infected with *Fusarium nivale* Ces. produced diseased plants. He states that not only *F. nivale* but other *Fusarium*s produce root diseases in cereal plants.

Bolley (6), from extensive field studies on wheat from land continuously cropped to wheat, has come to the conclusion that "through the practice of continuous wheating, soils in many cases have become infected with from one to three or four definite parasitic fungi which attack in the same manner as the flax-sick fungi attack and destroy the flax crop on flax lands and, therefore, such wheat lands may be said to be 'wheat sick.'" These views are further elaborated by him from extensive field studies and observations (7, 8). Bolley (9) also reports on the isolation of a considerable number of imperfect fungi from the nodes and internodes of wheat plants grown on experimental plats at the North Dakota Agricultural Experiment Station. Among them undetermined species of *Helminthosporium* and *Fusarium* occurred in abundance. No inoculation experiments are reported.

Schaffnit (29) in a comprehensive work on "Schneesimmel" gives a discussion of the fungus *Fusarium nivale* with relation to its occurrence, morphology, cultural characteristics, physiology, and preventive measures. He shows that this disease is due both to soil infection and seed infection, the former being more common. Incidental to his work on *F. nivale* Schaffnit (29) performed some inoculation experiments with *F. rubiginosum* Appel and Woll. on etiolated rye seedlings in damp atmosphere with positive results. The number of inoculations is not stated. *F. rubiginosum* has recently been demonstrated by Dr. H. W. Wollenweber to be identical with *F. culmorum*. A comprehensive bibliography of literature dealing with *Fusarium*s on cereals is given by Mortensen (16).

#### CONCLUSIONS

The experiments described in this paper and the literature cited show that some of the imperfect fungi occurring on small grains and inducing leaf spots or systemic infections are pathogenic when, under favorable conditions, they come in contact with seeds and seedlings,

while other forms apparently are nonparasitic. *Helminthosporium gramineum* and *Fusarium culmorum* were found to be parasitic, while *Cladosporium gramineum* and an undetermined species of *Altenaria* were not parasitic under the conditions here described. That only certain species are pathogenic is to be expected. Their identity as well as that of the large number of forms apparently saprophytic on cereals is more or less confused in the literature but should be determined, and the extent to which these fungi affect cereals should be ascertained by laboratory and greenhouse studies. These need to be reinforced by pure culture inoculations of seeds, seedlings, plants in various stages of growth, and soil under field conditions before the exact relation of such fungi to cereal cropping can be definitely established.

## LITERATURE CITED

1. APPEL, OTTO.  
1907. Fusarien als Erreger einer Fusskrankheit des Getreides. In Mitt. K. Biol. Anst. Land- u. Forstw., Heft 4, p. 32-33.
2. ———  
1908. Über die Schädigung von Getreide durch Fusarien. In Mitt. K. Biol. Anst. Land- u. Forstw., Heft 6, p. 10-11.
3. ———  
1909. Einige Krankheiten und Schädigungen des Wintergetreides. In Illus. Landw. Ztg., Jahrg. 29, No. 70, p. 665-666.
4. BECKWITH, T. D.  
1910. Mycological studies upon wheat and wheat soils to determine possible causes in deterioration in yield. In Science, n. s., v. 31, no. 803, p. 798.
5. ———  
1911. Root and culm infection of wheat by soil fungi in North Dakota. In Phytopathology, v. 1, no. 6, p. 169-176.
6. BOLLEY, H. L.  
1909. Deterioration in wheat yields due to root rots and blight producing diseases. N. Dak. Agr. Expt. Sta. Press Bul. 33, 4 p.
7. ———  
1911a. Interpretations of results noted in experiments upon cereal cropping methods after soil sterilization. In Science, n. s., v. 33, no. 841, p. 229-232.
8. ———  
1911b. The work of imperfect fungi in cereal crop deterioration. Abstract. In Science, n. s., v. 33, no. 842, p. 259-260.
9. ———  
1912. [Report on the work of the] Department of Botany and Plant Pathology. N. Dak. Agr. Expt. Sta. 22d Ann. Rpt., 1911/12, p. 23-60.
10. CHESTER, F. D.  
1891. The scab of the wheat. Del. Agr. Expt. Sta. 3d Ann. Rpt., 1890, p. 89-90, fig. 14-15.
11. DETMERS, FREDA.  
1892. Scab of wheat. In Ohio Agr. Expt. Sta. Bul. 44, p. 147-149, fig. 4-5.
12. EIDAM, E.  
1891. Das Vorkommen der Fleckenkrankheit auf Gersten- und auf Haferblättern. In Der Landwirt, Bd. 27, p. 509. Original not seen.
13. FRANK, A. B.  
1897. Kampfbuch gegen die Schädlinge unserer Feldfrüchte. 308 p., 46 fig., 20 pl. Berlin.

14. HECKE, L.  
1898. Die Braunfleckigkeit oder Blattbräune der Gerste. *In Wiener Landw. Ztg.*, Bd. 48, p. 435.
15. HILTNER, LORENZ, AND IHSEN, G.  
1911. Über das schlechte Auflaufen und die Auswinterung des Getreides infolge Befalls des Saatgutes durch Fusarium. *In Landw. Jahrb. Bayern*, Jahrg. 1, No. 1, p. 20-60, 8 fig.; No. 4, p. 315-362, 2 fig.
16. MORTENSEN, M. L.  
1911. Om Sygdomme hos Kornarterne, forarsagede ved Fusarium-Angreb (Fusarioser). *In Tidsskr. Landbr. Planteavl*, Bd. 18, p. 250.
17. MUTH, FRANZ.  
1908. Über die Infektion von Sämereien im Keimbett. Ein Beitrag zur Samenuntersuchung und Samenzüchtung. *In Jahresber. Ver. Angew. Bot.*, Jahrg. 5, 1907, p. 49-82.
18. PAMMEL, L. H.  
1892. New fungous diseases of Iowa. *In Jour. Mycol.*, v. 7, no. 2, p. 96-97.
19. ———, KING, CHARLOTTE M., AND BAKKE, A. L.  
1910. Two barley blights, with comparison of species of *Helminthosporium* upon cereals. *Iowa Agr. Expt. Sta. Bul.* 116, p. 179-190, 4 pl.
20. RAVN, F. K.  
1900. Nogle *Helminthosporium*-Arter og de af dem Fremkaldte Sygdomme hos byg og Havre. 220 p., illus., 2 pl. København.
21. RITZEMA BOS, J.  
1898. De bladplekziekte der gerst, veroorzaakt door *Helminthosporium gramineum* Rabhst. *In Landbouwk. Tijdschr.*, p. 42. Original not seen.
22. ———  
1900. Phytopathologisch laboratorium Willie Commelin Scholten. Verslag over de inlichtingen gegeven in 1899. *In Landbouwk. Tijdschr.*, p. 126. Original not seen.
23. ———  
1904-5. Geringe kiemkracht van in 1903 gewonnen zaad. *In Tijdschr. Plantenziekten*, jaarg. 10, afl. 5/6, p. 152-165, 1904; jaarg. 11, afl. 4/5, p. 124-137, 1905.
24. ———  
1905. Phytopathologisch laboratorium Willie Commelin Scholten. Verslag over onderzoekingen gedaan in—en over inlichtingen gegeven van wege hovenogenoemd laboratorium in het jarr 1904. *In Tijdschr. Plantenziekten*, jaarg. 11, afl. 1/2, p. 24-25.
25. ROSTRUP, E.  
1893. Oversigt over de i 1892 hos Markens Avlsplanter optraadte Sygdomme. *In Tidsskr. Landökon.*, Række 5, Bd. 12, p. 633-664. Original not seen.
26. ———  
1895. Oversigt over Sygdommenes Optraeden hos Landbrugets Avlsplanter i Aarets 1893. *In Tidsskr. Landbr. Planteavl*, Bd. 1, p. 140.
27. ———  
1903. Oversigt over Landbrugsplanternes Sygdomme i 1902. *In Tidsskr. Landbr. Planteavl*, Bd. 10, p. 364.
28. ———  
1904. Oversigt over Landbrugsplanternes Sygdomme i 1903. *In Tidsskr. Landbr. Planteavl*, Bd. 11, p. 402.
29. SCHAFFNIT, E.  
1913. Der Schneeschimmel und die übrigen durch *Fusarium nivale* Ces. hervorgerufenen Krankheitserscheinungen des Getreides. *In Landw. Jahrb.*, Bd. 43, Heft 4, pl. 1-4.

30. SELBY, A. D.  
1898. Some diseases of wheat and oats. Ohio Agr. Expt. Sta. Bul. 97, p. 40-42, fig. 4.
31. SELBY, A. D., MANNS, T. F.  
1909. Studies in diseases of cereals and grasses. Ohio Agr. Expt. Sta. Bul. 203, p. 212-224.
32. SORAUER, PAUL.  
1901. Der Schneeschimmel. In Ztschr. Pflanzenkrank., Bd. 11, Heft 4/5, p. 217-228.
33. ———  
1903. Über Frostbeschädigungen am Getreide und damit in Verbindung stehende Pilzkrankheiten. In Landw. Jahrb., Bd. 32, Heft 1, p. 1-68, 1 fig., pl. 1-4.
34. VOLKART, ALBERT.  
1908. Pflanzenschutz. In Landw. Jahrb. Schweiz, Jahrg. 22, p. 32-33.
35. WESTERDIJK, JOHANNA.  
1909. Fusarium in de tarwe. In Phytopath. Lab. "Willie Commelin Scholten," Jaarverslag. 1907/08, p. 3-4.

PLATE LXII

Fig. 1.—Wheat seedlings from seed inoculated with spores of *Helminthosporium gramineum* and from seed externally sterilized; photographed three weeks after planting. The three rows of pots on the left were sown with seed inoculated with spores of *H. gramineum* from barley, the two rows in the center with sterilized seed, and the three rows on the right with spores of *H. gramineum* from wheat.

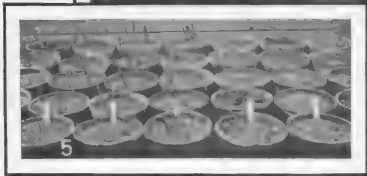
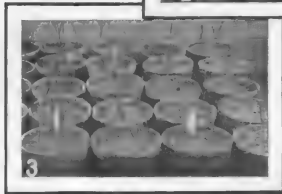
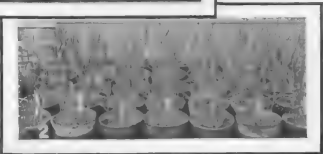
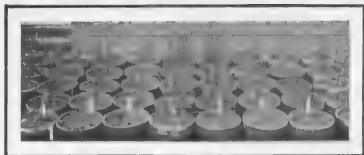
Fig. 2.—Barley seedlings from seed inoculated with *Helminthosporium gramineum* and from sterilized seed; photographed three weeks after planting. The three rows of pots on the left from seed inoculated with spores of *H. gramineum* from barley, the next three rows from seed externally sterilized, and the row on the right from seed inoculated with *H. gramineum* from wheat.

Fig. 3.—Wheat seedlings from seed inoculated with spores of *Fusarium culmorum* from oat seedlings (two rows of pots on right) and from seed externally sterilized (two rows of pots on left). Photographed two weeks after planting.

Fig. 4.—Barley seedlings from seed inoculated with spores of *Fusarium culmorum* from oat seedlings (two rows of pots on right) and from seed externally sterilized (three rows on left). Photographed two weeks after planting.

Fig. 5.—Oat seedlings from seed inoculated with spores of *Fusarium culmorum* from oat seedlings (two rows on right) and from seed externally sterilized (three rows on left). Photographed two weeks after planting.





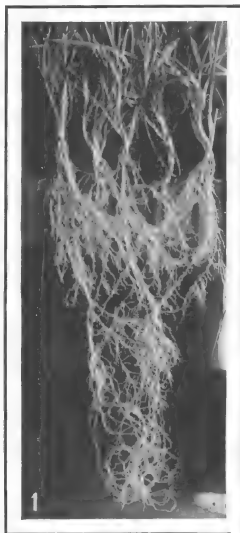


PLATE LXIII

Root systems of wheat seedlings grown in 6-inch pots from seed externally sterilized (left) and from seed inoculated with *Helminthosporium gramineum* from wheat (right). Photographed six weeks after planting.

# THE ORIGIN OF SOME OF THE STREPTOCOCCI FOUND IN MILK

By L. A. ROGERS and ARNOLD O. DAHLBERG,  
*Dairy Division, Bureau of Animal Industry*

## INTRODUCTION

In the higher plants and animals we are accustomed to associating species with a more or less definite habitat. Certain animals are found only in certain localities. One species of trees may be found only on a particular type of soil. A still narrower limit of distribution is found in some of the parasitic fungi which grow only on closely related host plants. Zoologists or botanists find the types on which they base their descriptions in the natural habitat of the organism. This relation has not always existed in the published descriptions of bacteria. The association of a natural group with a particular habitat has been more or less incidental, except perhaps with the pathogenic bacteria, and even with some of these it is not impossible that the pathological conditions under which they are found may not be the true habitat of the species. The colon group, while it is frequently found in water and milk, has its natural habitat in the intestinal tract of warm-blooded animals. Winslow found that certain chromogenic cocci were associated with the skin of animals.<sup>1</sup> Some of the English bacteriologists have pointed out that the streptococci from horse manure, for instance, have a set of physiological reactions which differentiates them from those from saliva or pathological conditions.<sup>2</sup> It is only through a knowledge of the habitat and the study of sufficient cultures to establish a type that true bacterial species can be determined. If we were to write a description of the German people we would go to Germany, not to an American city where German immigrants live.

Countless descriptions have been written of bacteria isolated from milk until we have come to consider certain types as peculiar to this medium. The bacteria found in milk, however, are a heterogeneous collection, and the true types of milk bacteria are to be sought in the sources from which milk is contaminated. Esten has suggested that the streptococci or lactic-acid bacteria of milk come originally from the mouth of the cow.<sup>3</sup> The feces of the animal must, unfortunately,

---

<sup>1</sup> Winslow, C. E. A., and Winslow, Anne R. *Systematic relationships of the Coccaceæ*. ed. 1, 300 p., illus. New York, 1908.

<sup>2</sup> Andrewes, F. W. Report on the micro-organisms present in sewer air and in the air of drains. 36th Ann. Rpt. Local Govt. Bd. [Gt. Brit.], 1906-07, Suppl. Rpt. Med. Off., p. 183-204. 1908.

<sup>3</sup> Esten, W. M. *Bacterium lactis acidii* and its sources. Conn. Storrs Agr. Expt. Sta. Bul. 59, 27 p., 5 fig. 1909.

be considered as a possible source of bacteria in milk, among which would undoubtedly be found members of the lactic group. Kinyoun and Dieter believe that the presence in milk of cocci which form chains in lactose bile at 37° C. is presumptive evidence that the milk is contaminated with feces.<sup>1</sup> It is the more common practice, however, to consider this type as the indication of the presence in the herd producing the milk of one or more cows with infected udders.

The mouth is known to contain streptococci, and the habit of cows of licking their flanks and udders provides a more or less direct connection between the mouth and the milk pail. Each of these sources may be considered as the normal habitat of bacteria. Under these conditions they persist for indefinite generations, adapting themselves to their environment until it is reasonable to suppose the characters acquired become sufficiently fixed to have at least varietal significance.

The study of streptococci originating within such circumscribed limits is of interest in addition to its taxonomic importance, in the light it may cast on the origin of some of the bacteria in milk and the significance from the hygienic standpoint of the presence of certain types.

In this paper are recorded the results of a study of streptococci representing three of the possible sources from which this group may find its way into milk. The morphology of this collection was studied with the hope that this would give some basis for a division into varieties. The ability of these cultures to utilize a number of carbohydrates and alcohols was determined. On the basis of these fermentations several groups are established, each of which is made up of a large number of identical cultures constituting the type about which are grouped similar cultures, but which varied from it in one or two reactions. The probable relation of one of these groups to well-known species is pointed out.

#### THE CULTURES STUDIED

A collection of streptococci were obtained from milk, from bovine feces, from the mouths of cows, and from the udders of cows. With the exception of those from milk an effort was made to make the cultures as representative as possible. The procedure of isolating the milk cultures followed that usually employed in the laboratories of boards of health. Small portions of the milk were added to lactose-bile tubes which were incubated at 37° C. Tubes showing streptococci in distinct chains on microscopical examination were plated on lactose agar and the chain-forming cocci subcultured. In this way 42 cultures were isolated from 25 samples of milk and cream collected at Washington or at the creamery at Troy, Pa. No two samples came from the same farm. A few cultures were obtained through the courtesy of Dr. Kinyoun and Mr. Dieter from lactose-bile tubes in the laboratory of the health department of the District of Columbia. These cultures, therefore, did not

<sup>1</sup> Kinyoun, J. J., and Dieter, L. V. A bacteriological study of the milk supply of Washington, D. C. *Jour. Amer. Pub. Health Assoc.*, v. 2, no. 4, p. 262-274. 1912.

represent the normal streptococci of milk but rather those which would usually be distinguished as indicating contamination from infected udders or fecal sources.

Fifty-one cultures were isolated from 19 samples of milk obtained by milking directly into sterile test tubes. The cows from which these samples were obtained represented all gradations of infected udder from occasional evidence of garget to acute mammitis. Part of these were in the Dairy Division herd at Beltsville, Md., and the remainder in the herd on the Naval Academy farm at Annapolis, Md. One hundred and fourteen cultures came from 56 samples of cow manure obtained, with the exception of a few from Troy, Pa., at the Dairy Division farm and at the dairy of the Government Hospital for the Insane at Washington. Thirty-nine cultures were made from the mouths of animals at the Dairy Division farms. With the exception of one culture obtained from the mouth of a mule, all of these cultures were of bovine origin. In Table II the origin of the culture is indicated by M for milk, U for udder, F for feces, and B for mouth. The sample from which the culture was secured is indicated by a number following the letter. For instance, "F15" represents sample of feces No. 15. This will enable the reader to determine the origin of each culture and the number of cultures from each sample.

#### MORPHOLOGY OF THE CULTURES

While it is generally recognized that there is little morphological basis for subdivisions of the streptococci, reference is frequently made to certain types of cells. Stowell, Hilliard, and Schlesinger,<sup>1</sup> in selecting streptococci from milk for comparison with those isolated from the human throat, rejected diplococci and the oval-chained form which they designate as the *Streptococcus lacticus* of Kruse or the *Bacillus lactis acidi* group, respectively. In selecting our cultures no attention was paid to morphology beyond determining that it was a coccus apparently dividing in one plane, with the exception of those from milk, which were not accepted if they did not form chains of at least 8 or 10 cells. The morphology of nearly all cultures was determined by examination of specimens stained with gentian violet. Camera-lucida drawings were made using a Leitz 3 mm. objective and No. 18 ocular, a combination which gave a magnification of 2,400 diameters at the ocular, or 4,800 diameters on the drawing board. Sufficient light to give a clear image was obtained by using a special arc light with a copper-sulphate ray filter.

Preliminary studies showed that the medium on which the culture was grown had an appreciable influence on both the size and the form of the cell. This is shown in figure 1, which is reproduced from camera-lucida drawings of typical cultures grown on various media. Milk gave quite

<sup>1</sup> Stowell, E. C., Hilliard, C. M., and Schlesinger, M. J., A statistical study of the streptococci from milk and from the human throat. Jour. Infect. Diseases, v. 12, no. 2, p. 144-164. 1913.

uniformly smaller cells and less tendency to chain formation than broth or agar. The cells at *a* are from culture *lo* on milk, *b* on broth, and *c* on agar, all incubated 48 hours at 37° C. The difference between the distinctly rod-shaped cell found on agar and the small round cell obtained from milk is marked. That differences in size of cells are not due entirely to differences in the medium is shown by the chain at *h*. This combination of small and large cells in a single chain is not unusual in broth, a medium in which there is a marked tendency to form enlarged and abnormal cells. In some cultures the transition from normal cells to those of monstrous size and form was so rapid that it was difficult to

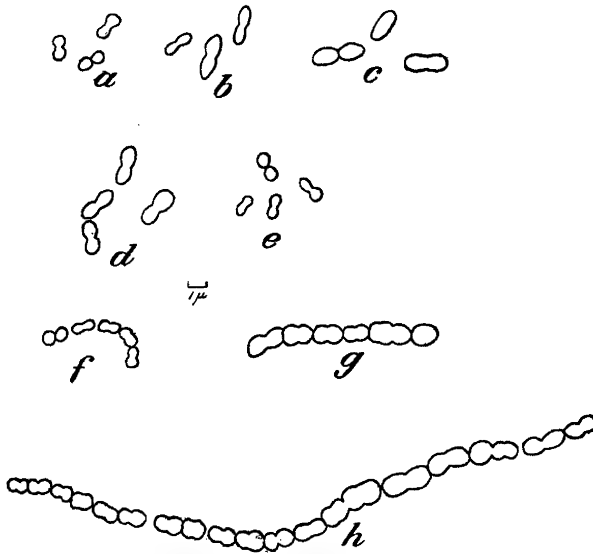


FIG. 1.—Cells of streptococci, showing variation in size and morphology. *a*, culture *lo* on milk; *b*, culture *lo* on broth; *c*, culture *lo* on agar; *d*, culture *li* on lactose bile; *e*, culture *li* on broth; *f*, culture *gm* on milk; *g* and *h*, culture *gm* on broth.

Various types of cells which were found in this collection are shown in figure 2. It will be observed that much of the variation in these types is in size only or in chain formation. The slender-pointed cells shown at *F* were peculiar to the cultures obtained from the mouth of animals, but the cultures from this source were not confined to this type. In Table II the letter under the heading "Morphology" refers to figure 2, although it is obvious that in many cases the assignment to a particular type can be only an approximation. The variation of the morphology is so great and so easily affected by the environment that it was not considered in the final arrangement of groups. It should be stated, however, that among the udder cultures the tendency to chain formation was much more marked and more constant than among all other cultures.

#### METHODS OF DIFFERENTIATION

When morphological distinctions are lacking, we are forced to use the physiology of the organism as a basis of classification. No single system

obtain preparations showing what could be considered normal cells. The most satisfactory preparations were obtained in incubating broth cultures until a distinct cloudiness was obtained, centrifuging the culture, siphoning off the broth, and washing the sediment with sterile water. After centrifuging again the water was siphoned off, and a preparation made from the sediment. This gave a clear field suitable for examination under a high-power microscope.

of characters can be adopted for all classes of bacteria. The significant characters will be found for each group only by a study of its normal activities and the utilization of those functions which show the nature, limitations, and relationship of the group. The striking characteristic of the streptococci is their ability to form acids from carbohydrates and related substances, and this peculiarity has been very generally utilized for purposes of classification. The voluminous literature bearing pro and con on the constancy and the value of these tests has been reviewed fully in various papers and need not be taken up here. It may be safely asserted, however, that the fermentative ability is as constant and as significant for purposes of classification as the characters adopted by those who reject the fermentation tests as too variable. For instance, Davis, who rejects the sugar fermentations as untrustworthy, divides the

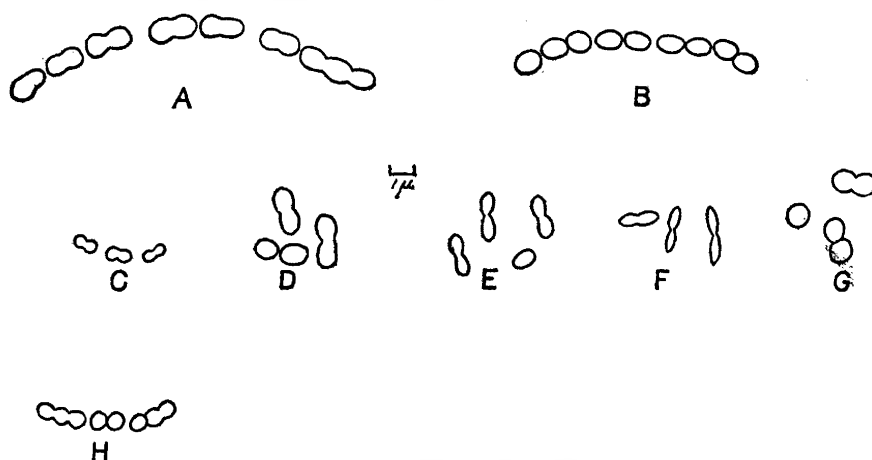


FIG. 2.—Types of cells of streptococci.

streptococci into five groups on the basis of hemolysis, green colonies on blood agar, capsule, solubility in bile, inulin fermentation, experimental arthritis, and experimental endocarditis.<sup>1</sup>

For purposes of classification, we have used the liquefaction of gelatin and the fermentation of dextrose, saccharose, lactose, raffinose, starch, inulin, mannite, and glycerin. Adonite and dulcite were tested, but as they were fermented by only one or two of these cultures they were of no value. The liquefaction of gelatin was determined by inoculating the surface of the gelatin tube with a few drops of a broth culture and measuring the liquefaction after 30 days at 20° C.

The fermentation of the test substances was determined in a medium made as follows:

	Per cent.
Beef extract.....	0.4
Peptone.....	1.0
Dibasic potassium phosphate.....	.5
Test substance.....	2.0

<sup>1</sup> Davis, D. J. Interrelations in the streptococcus group with special reference to anaphylactic reactions. Jour. Infect. Diseases, v. 12, no. 3, p. 386. 1913.



The cultures were incubated for seven days at 30° and titrated cold against twentieth-normal sodium hydrate with phenolphthalein as an indicator. From the results so obtained is subtracted the titration of a blank, and the result is expressed as the percentage of normal acid. Some objection may be raised against the use of 30° C. as an incubation temperature rather than the more common one of 37°. The lower temperature was adopted because practically all streptococci will grow at this temperature, while a few grow at 37° slowly or not at all.

The fermentation produced by the streptococci is in almost all cases so marked that there is very rarely any question about the presence or

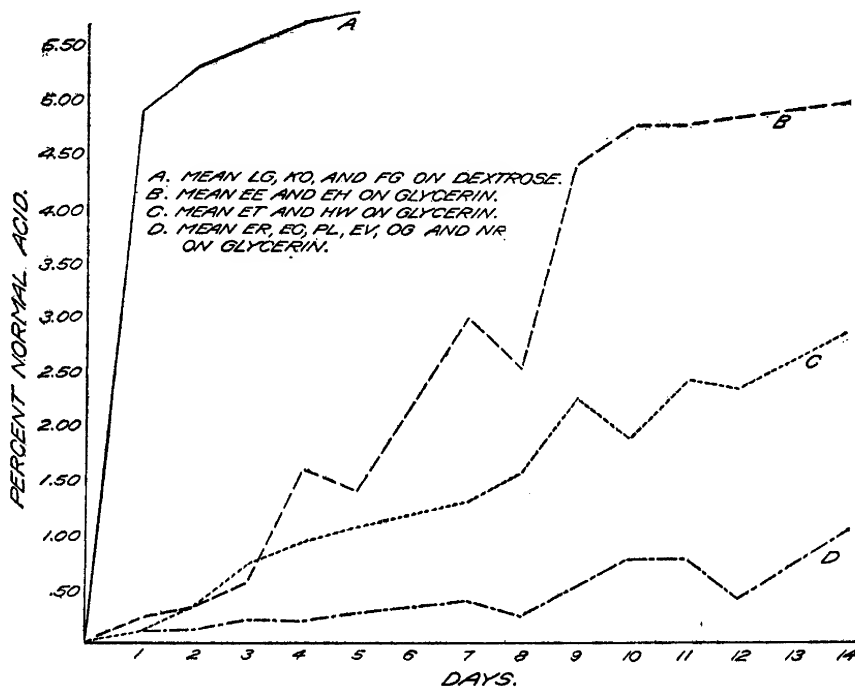


FIG. 3.—Curve showing the typical rate of fermentation of dextrose and glycerin.

absence of the fermentation. Of all the substances we have used glycerin forms an exception to this rule. The fermentation proceeds slowly and in seven days may be slightly above or slightly below 1 per cent normal acid, the point selected as marking the line between fermentation and no fermentation. This is illustrated by Table I, which shows the progressive rate of fermentation by typical cultures. Three cultures fermenting dextrose are included to show the usual course of the fermentation in the more easily fermented sugars. Each titration was made from a separate tube. A study of this table shows that the 12 cultures may be divided into three quite distinct types on the basis of the rate of fermentation of glycerin. This is shown more clearly in figure 3, in which the average titrations for each of the three types are plotted. Two of these cultures fermented the glycerin with comparative

rapidity and after three days there was no question that the cultures were able to utilize glycerin. Those represented by the curve *D*, on the other hand, produce only a very slight increase in acidity, which even at the end of 14 days is only slightly above 1 per cent normal.

Between these two is a third group in which there is a slow but distinct increase in acidity. At seven days the acidity is above 1 per cent normal. While an error may be introduced in some cases by drawing the line between fermentation and no fermentation of glycerin at 1 per cent normal, it is believed that in these results this error will be slight. These results illustrate the value of the exact results obtained by titration which we have always used in preference to the simpler and more rapid way of determining the change of reaction with litmus in solution in the broth or with litmus papers.

We also consider it a decided advantage to allow sufficient time for the completion of the fermentation, thus securing an end point rather than some intermediate and varying determination. Seven days are not sufficient for the completion of the glycerin fermentation, but it is undoubtedly ample for other test substances which we have used.

TABLE I.—*Progressive fermentation of dextrose and glycerin.*

DEXTROSE.													
Culture No.	Percentage of normal acid produced in—												
	1 day.	2 days.	3 days.	4 days.	5 days.	7 days.	8 days.	9 days.	10 days.	11 days.	12 days.	14 days.	
lg.....	4.6	5.2	5.2	5.6	5.5	.....	.....	.....	.....	.....	.....	.....	.....
ko.....	4.6	5.0	5.2	5.5	5.5	.....	.....	.....	.....	.....	.....	.....	.....
fg.....	5.2	5.8	6.2	6.1	6.5	.....	.....	.....	.....	.....	.....	.....	.....
GLYCERIN.													
ec.....	0.15	0.19	0.31	0.34	0.44	0.55	0.58	0.91	0.53	0.96	0.78	1.21	
ee.....	.25	.23	.80	1.67	1.31	3.65	2.73	4.96	4.85	4.96	.....	5.43	
eh.....	.22	.46	.35	1.57	1.54	2.40	2.38	3.96	5.06	4.61	3.74	4.68	
er.....	.15	.14	.37	.37	.96	.86	.28	.63	1.83	.92	.33	1.13	
et.....	.05	.59	.82	.95	1.09	1.30	1.49	2.16	1.73	2.18	2.30	2.63	
ev.....	.15	.09	.34	.17	.00	.40	.45	.66	1.03	.96	.70	1.18	
hw.....	.20	.11	.67	.95	1.07	1.35	1.67	1.38	2.08	2.71	2.45	3.15	
nr.....	.15	.09	.14	.00	.00	.25	.02	.31	.43	.76	.21	.....	
ny.....	.20	.29	.04	.00	.05	.03	.00	.01	.00	.21	.00	.....	
og.....	.11	.09	.02	.07	.09	.05	.02	.11	.48	.46	.03	.....	
om.....	.15	.00	.09	.11	.09	.06	.00	.21	.13	.19	.00	.00	
pl.....	.05	.09	.22	.32	.24	.36	.31	.71	.53	.76	.66	.78	

#### THE CORRELATION OF PHYSIOLOGICAL CHARACTERS

The complete results of the tests made on this collection of cultures are presented in Table II. The reduction of neutral red and the curdling of milk are given in the table, but are not used in the correlations. Adonite and dulcite are necessarily excluded, since these cultures almost without exception fail to ferment these two substances.

TABLE II.—Physiological characters of all cultures.

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid.											
			Milk.	Broth.				Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.		
ec	M <sub>1</sub>	G	.....	—	mm.	.....	+	4.1	0	0.1	4.3	0	1.3	0	0	0.6	0.1		
ed	U <sub>1</sub>	G	.....	—	30	+++	+	1.7	0.1	4.0	4.0	0	4.4	0	2.4	0.8	0.1		
ee	U <sub>1</sub>	G	.....	—	28	+++	+	2.5	0	4.1	4.2	0.1	2	0.2	2.9	0.8	0.1		
eh	U <sub>1</sub>	G	.....	+	27	+++	+	2.6	0	3.6	3.8	0.2	2	0	1.8	0.9	0		
ej	U <sub>2</sub>	B	.....	+	.....	+++	+	2.7	0	2.7	4.6	0	0	0.1	0	1.1	0.1		
en	M <sub>2</sub>	G	.....	—	0	+++	+	4.4	0	0	4.5	0.1	2	0	2.3	0.6	0		
eo	M <sub>3</sub>	G	.....	—	50	+++	+	4.3	0.1	4.7	5.1	0.2	4	0.5	4.4	1.7	0.2		
eq	M <sub>4</sub>	G	.....	—	0	+++	+	4.1	0.1	4.8	4.8	0	2	0.1	4.0	2.1	0.1		
er	M <sub>5</sub>	G	.....	—	0	+++	+	4.6	0	2	5.4	0	2	0	1	0.7	0		
et	M <sub>6</sub>	G	.....	—	0	+++	+	6.5	0	4.1	5.1	0.1	1	0.1	3.9	0.9	0		
eu	M <sub>7</sub>	G	.....	—	0	+++	+	6.6	0	4	5.0	0	0	0	2.4	0.4	0		
ew	M <sub>7</sub>	G	.....	—	0	+++	+	3.0	0	3	5.0	0	0	0.1	2.1	0.3	0		
ez	U <sub>1</sub>	G	.....	—	0	+++	+	4.9	0	3.9	3.6	0	0	0	1.5	0.7	0		
fa	U <sub>1</sub>	G	.....	—	19	+++	+	4.7	0	3.0	3.6	0.1	1	0	0.9	0.6	0		
fc	U <sub>2</sub>	D	.....	—	55	+++	+	2.0	0.2	3.4	3.2	0.2	3	0.4	0.8	0	0.2		
fg	U <sub>2</sub>	D	.....	—	0	+++	+	1.8	0	0	4	0	0	0	0	0.2	0.2		
fi	M <sub>8</sub>	E	.....	—	49	+++	+	6.6	0	4	5.6	0	3	0.2	6.2	2.0	0.1		
fj	M <sub>9</sub>	C	.....	—	0	+++	+	5.3	0	5.6	5.6	0.2	1	0	3.9	0	0.1		
fk	M <sub>10</sub>	C	.....	—	0	+++	+	5.6	0.2	5.3	4.9	0.1	0	0	3.5	0	0		
fl	M <sub>11</sub>	C	.....	—	0	+++	+	7.1	0	2	5.7	0.3	0	0.1	0	0.5	0.2		
fm	M <sub>12</sub>	C	.....	—	0	+++	+	5.0	0	4	5.2	0.4	2	0.2	4.3	1.3	0.1		
fo	M <sub>12</sub>	G	.....	—	0	+++	+	5.1	0	5.5	5.3	0	2	0	4.2	0	0		
fp	M <sub>12</sub>	G	.....	—	0	+++	+	5.5	0.1	5.6	4.5	0	0	0	4.4	0.1	0		
fr	M <sub>13</sub>	G	.....	—	0	+++	+	5.9	0.1	5.8	4.7	0	2	0	4.3	0.4	0		
ft	M <sub>13</sub>	G	.....	—	0	+++	+	5.3	0	4.8	5.5	0.3	0	0.1	4.3	1.2	0.1		
fu	M <sub>9</sub>	G	.....	—	0	+++	+	5.4	0	5.1	5.3	0	1	0.1	2.7	0.2	0.1		
fv	M <sub>10</sub>	G	.....	—	0	+++	+	7.0	0	1	5.2	0	0	0	0.1	0.5	0		
fw	M <sub>14</sub>	C	.....	—	0	+++	+	6.6	0	1	5.6	0.1	1	0.1	0	0.5	0		
fx	M <sub>15</sub>	C	.....	—	0	+++	+	5.4	0	4.8	5.2	0.2	1	0.1	4.1	1.2	0.1		
fy	M <sub>15</sub>	C	.....	—	0	+++	+	5.3	0	5.4	4.6	0	0	0	3.8	0.1	0		
ga	M <sub>15</sub>	C	.....	—	0	+++	+	5.3	0	5.5	5.2	0.2	1	0	4.3	1.1	0		
gb	M <sub>16</sub>	C	.....	—	0	+++	+	6.0	0	0	5.6	0.2	0	0	1	0.1	0		
gc	U <sub>3</sub>	H	.....	+	0	+++	+	6.5	0.1	1	5.4	0	1	0.2	1	0.4	0.1		
gd	U <sub>3</sub>	H	.....	+	0	+++	+	4.4	0	3.6	4.0	0	1	3.8	0	0	0		
ge	U <sub>4</sub>	H	.....	+	0	+++	+	4.6	0	4.7	4.8	0.3	4	0	0	0.1	1.9		
gh	U <sub>4</sub>	H	.....	+	0	+++	+	4.2	0	0	4.9	0.1	0	0.1	0	0.1	0		
gi	U <sub>5</sub>	B	.....	+	0	+++	+	4.8	0	1	4.3	0	2	0	0	0.1	0		
gk	U <sub>5</sub>	B	.....	+	0	+++	+	4.5	0.1	4.4	4.8	0.1	0	0.1	0	0.3	0		
gl	U <sub>5</sub>	B	.....	+	0	+++	+	4.6	0	0	4.3	0	0	0	0.2	0	0		
gm	U <sub>5</sub>	A	.....	+	0	+++	+	4.4	0.1	4.7	4.9	0.2	0	0	0	0	0		
go	U <sub>6</sub>	A	.....	+	0	+++	+	4.5	0	4.8	4.8	0	0	0	0	0	0		
gp	U <sub>5</sub>	A	.....	+	0	+++	+	4.3	0.3	0	4.2	0	0	0.7	0	0.1	0		
gt	U <sub>5</sub>	A	.....	+	0	+++	+	4.7	0.3	4.6	4.6	0.1	4	0	0	0.3	0		
gw	U <sub>6</sub>	A	.....	+	0	+++	+	4.5	0	4.6	5.0	0	1	0	0	0	0.1		
gx	U <sub>2</sub>	B	.....	+	0	+++	+	4.5	0.1	4.9	5.0	0.1	0	0.2	0	0	0		
gz	U <sub>6</sub>	B	.....	+	0	+++	+	4.7	0	0	4.4	0	0	0	0	0	0		
ha	M <sub>17</sub>	E	.....	—	0	+++	+	6.4	0	0	5.1	0.1	0	0	2.3	0.3	0		
hb	M <sub>17</sub>	E	.....	—	0	+++	+	6.4	0.2	0	5.1	0.1	0	0.1	3.8	0.4	0.1		
hc	M <sub>17</sub>	E	.....	—	0	+++	+	5.5	0.1	0	5.9	0.1	2	0	0.2	0.5	0		
hd	M <sub>18</sub>	E	.....	—	0	+++	+	5.8	0	5.6	5.2	0	0	0.1	3.9	0.4	0		
he	M <sub>18</sub>	E	.....	—	0	+++	+	6.1	0	5.5	5.6	0.1	0	0	3.4	0.4	0.4		
hg	M <sub>19</sub>	E	.....	—	0	+++	+	5.9	0.2	2	5.5	0.1	1	0.1	0.2	0.7	0		
hh	M <sub>20</sub>	E	.....	—	0	+++	+	6.2	0.1	0.1	5.5	0.1	2	0.2	0	0.6	0		
hj	M <sub>20</sub>	E	.....	—	0	+++	+	6.8	0	0	4.7	0.1	2	0	0	0.4	0		
hk	M <sub>21</sub>	E	.....	—	0	+++	+	4.3	0.2	2	5.5	0.1	1	0	0	0.5	0.1		
hl	M <sub>21</sub>	E	.....	—	0	+++	+	7.1	0.2	0	5.6	0	0	0	0	0.5	0		
hm	M <sub>22</sub>	E	.....	—	0	+++	+	6.1	0.4	0	5.3	0.1	1	0.1	2.8	0.3	0		
hn	M <sub>22</sub>	E	.....	—	0	+++	+	5.6	0.2	0	6.3	0.1	1	0	2.7	0.4	0		
ho	F <sub>2</sub>	E	.....	—	0	+++	+	5.7	0	6.0	5.5	5.3	0	0.1	0	0	0		
hq	F <sub>3</sub>	E	.....	—	0	+++	+	6.4	0.2	5.5	5.3	0	0	0	0.1	0.1	0		
hr	F <sub>3</sub>	E	.....	—	0	+++	+	4.7	0	5	5.0	0.1	0	0.1	0	0	0.1		
hs	F <sub>4</sub>	E	.....	—	0	+++	+	5.8	0.3	5.8	5.7	5.3	0	0	0	0.1	0		
hu	M <sub>23</sub>	E	.....	—	0	+++	+	6.0	0.1	5.5	5.2	0.3	3	0	4.6	0	0		
hv	M <sub>23</sub>	E	.....	—	0	+++	+	6.2	0.7	5.1	7	0	0	0	5.0	1.5	0.1		
hw	M <sub>24</sub>	E	.....	—	0	+++	+	5.3	0.3	4.8	5.1	0.2	2	0	4.3	0.5	0		
hx	M <sub>24</sub>	E	.....	—	0	+++	+	5.0	0.1	4.7	5.2	0.2	2	0.1	4.1	1.5	0		
hy	F <sub>5</sub>	E	.....	—	0	+++	+	5.7	0.2	5.9	5.5	5.3	4.7	0	0	0	0.1		
hz	F <sub>5</sub>	E	.....	—	0	+++	+	5.1	0	5.4	5.1	5.0	4.7	0	0	0	0		
ib	F <sub>6</sub>	E	.....	—	0	+++	+	5.8	0.2	6.4	5.8	5.1	0	0	4.3	0.2	0		
ic	F <sub>6</sub>	E	.....	—	0	+++	+	5.7	0	6.7	5.6	5.2	0	0	4.3	0.2	0		
id	F <sub>7</sub>	E	.....	—	0	+++	+	5.7	0	5.8	5.6	5.4	0	0	0	0.1	0		
ie	F <sub>7</sub>	E	.....	—	0	+++	+	5.7	0	6.0	5.4	5.3	0	0	0	0	0		
if	F <sub>8</sub>	E	.....	—	0	+++	+	4.9	0	5.5	5.4	0	0	0	0	0	0.1		
ig	F <sub>8</sub>	E	.....	—	0	+++	+	4.3	0	4.6	5.2	5.9	0	0.1	0	0	0		
ih	F <sub>9</sub>	G	.....	—	0	+++	+	6.1	0.2	5.5	4.8	1.8	0	0	1.7	0.2	0		
ii	F <sub>9</sub>	G	.....	—	0	+++	+	7.7	0.1	8.9	5.4	3.2	0.2	0	1.4	0.3	0		

TABLE II.—Physiological characters of all cultures—Continued.

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid.										
			Milk.	Broth.				Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.	
ik	M <sub>25</sub>	G	—	—	mm.	—	+	5.2	0.2	4.3	4.7	3.2	0.2	2.9	4.0	0.2	0.1	
il	M <sub>25</sub>	E	—	—	0	—	+	4.6	0.2	4.4	4.7	3.5	0.2	3.2	3.7	0.3	0.1	
im	F <sub>10</sub>	—	—	—	0	—	+	6.5	0	6.1	5.0	4.8	4.6	0	0.3	0.1	0	
io	F <sub>11</sub>	—	—	—	0	—	+	4.8	0.2	5.5	5.5	5.1	4.4	0	0	0	0	
ip	F <sub>12</sub>	—	—	—	0	—	+	5.8	0	5.8	5.1	5.0	0.2	0	0	0.3	0	
ir	F <sub>13</sub>	E	—	—	0	—	+	5.4	0.1	5.4	5.6	5.0	0.1	0	0	0.2	0	
is	F <sub>13</sub>	—	—	—	0	—	+	5.6	0.2	7.6	5.7	4.5	0.3	0	1.8	0.2	0	
it	F <sub>13</sub>	—	—	—	0	—	+	4.8	0.1	5.6	5.9	5.9	4.4	0	0	0	0	
iu	F <sub>15</sub>	E	—	—	0	—	+	5.5	0	5.7	5.9	6.0	4.7	0.1	0	0	0.1	
iv	F <sub>5</sub>	—	—	—	0	—	+	5.5	0.1	5.3	4.9	4.8	6.2	0	0	0.4	0	
ix	F <sub>14</sub>	—	—	—	0	—	+	6.1	0.2	6.2	5.4	0.1	0	0	5.3	0.8	0	
jd	F <sub>15</sub>	D	—	—	0	—	+	5.5	0.1	6.2	5.8	5.1	4.5	0	4.1	0.2	0	
je	F <sub>15</sub>	—	—	—	0	—	+	6.9	0	6.0	5.2	4.7	6.2	0	0	0	0	
jj	F <sub>15</sub>	E	—	—	0	—	+	4.3	0.1	5.4	5.1	5.0	0	0	0	0	0	
jg	F <sub>15</sub>	—	—	—	0	—	+	5.6	0.2	6.3	5.5	5.3	0	0	0	0	0	
jh	F <sub>16</sub>	—	—	—	0	—	+	5.7	0.1	5.8	5.5	5.0	4.4	0	0	0	0	
ji	U <sub>7</sub>	—	+	—	0	—	+	4.5	0.2	4.2	5.1	0	0.1	0	0	0	0.1	
jk	U <sub>7</sub>	—	+	—	0	—	+	4.5	0.1	4.6	4.9	0	0	0	0	0	0	
jl	U <sub>2</sub>	—	—	—	0	—	+	4.7	0	4.4	4.6	0	0	0.1	0	0	0	
jm	U <sub>5</sub>	—	+	—	0	—	+	4.8	0.1	4.9	4.4	0.1	0	0.1	0	0	0.1	
jn	U <sub>5</sub>	—	—	—	0	—	+	3.4	0.1	4.8	4.5	0.3	0.1	0.2	0	0.2	0	
jo	U <sub>3</sub>	H	+	+	0	—	+	4.8	0	4.5	3.6	0.2	0.3	3.9	2.7	0.2	0	
jp	U <sub>8</sub>	—	—	—	0	—	+	4.8	0.1	3.8	0	0	0.1	0.1	3.6	0	0	
jr	U <sub>8</sub>	—	—	—	0	—	+	4.5	0.1	3.8	4.0	0	0	0	3.4	0.1	0.1	
js	F <sub>17</sub>	D	—	—	0	—	+	6.4	0.2	6.2	4.9	4.9	0	0	0.3	0	0	
jt	F <sub>17</sub>	—	—	—	0	—	+	5.5	0.1	5.8	5.3	5.2	0	0.1	0	0.1	0	
ju	F <sub>17</sub>	G	—	—	0	—	+	5.4	0.2	5.9	5.3	5.1	4.8	0	0.2	0.1	0	
jv	F <sub>18</sub>	E	—	—	0	—	+	5.8	0.1	4.9	5.2	4.9	3.9	6.4	0.1	0.1	0	
jw	F <sub>18</sub>	—	—	—	0	—	+	4.9	0	5.1	4.9	5.5	0.1	0.2	0.1	0	0.1	
jx	F <sub>18</sub>	—	—	—	0	—	+	5.9	0	4.8	4.9	5.1	6.0	5.8	0	0	0.1	
iy	F <sub>19</sub>	—	—	—	0	—	+	3.5	0	5.3	5.4	6.0	0.2	0	0.3	0.1	0	
jz	F <sub>20</sub>	—	—	—	0	—	+	5.4	0.2	5.8	5.1	5.1	0.1	0	0	0.2	0	
ka	F <sub>20</sub>	E	—	—	0	—	+	5.8	0	5.8	5.5	5.2	0	0	0	0	0	
kb	F <sub>20</sub>	—	—	—	0	—	+	6.9	0	0	5.5	0	0.2	0.1	0.1	0	0.1	
kc	F <sub>21</sub>	—	—	—	0	—	+	5.5	0.1	4.9	5.3	4.8	4.6	0.1	0	0.1	0	
kd	F <sub>21</sub>	—	—	—	0	—	+	5.4	0.1	5.8	5.0	5.1	4.6	0	0	0	0	
ke	F <sub>21</sub>	—	—	—	0	—	+	6.1	0.1	5.0	5.1	4.6	0.1	0	0	0	0	
kf	F <sub>22</sub>	—	—	—	0	—	+	5.9	0.1	6.1	6.2	0	0.2	0.1	3.9	0.1	0	
kg	F <sub>23</sub>	C	—	—	0	—	+	6.1	0.1	6.5	5.2	4.8	0.1	0	0	0	0	
kh	F <sub>23</sub>	—	—	—	0	—	+	5.9	0.1	5.9	5.0	5.3	0	0	0	0	0	
ki	F <sub>24</sub>	C	—	—	0	—	+	6.6	0.2	0	5.0	4.4	6.3	0	0.1	0	0	
kl	F <sub>24</sub>	—	—	—	0	—	+	5.8	0.1	5.9	5.2	4.3	0.1	0	4.0	0	0	
km	F <sub>24</sub>	H	—	+	0	—	+	6.6	0.1	6.0	5.3	4.7	0	0.2	4.1	0	0	
kn	F <sub>25</sub>	—	—	—	0	—	+	5.9	0.1	5.5	5.5	5.2	4.3	0.1	0	0.2	0	
ko	F <sub>25</sub>	C	—	—	0	—	+	4.9	0	5.2	4.3	4.8	0.1	0.1	4.1	2.3	0.1	
kp	F <sub>25</sub>	—	—	—	0	—	+	6.5	0.2	5.4	4.9	4.8	6.2	0	0.1	0	0	
kq	F <sub>26</sub>	—	—	—	0	—	+	5.8	0.2	5.9	5.4	5.1	0	0	0	0.1	0	
kr	F <sub>26</sub>	E	—	—	0	—	+	6.1	0.2	6.4	5.4	4.6	6.5	0	0	0.1	0	
ks	F <sub>26</sub>	D	—	—	0	—	+	5.8	0.2	6.1	5.5	5.1	0	0	0	0.1	0.1	
kt	F <sub>27</sub>	D	—	—	0	—	+	7.3	0.2	5.9	5.7	0.2	0.2	0	2.2	0.3	0.1	
ku	F <sub>27</sub>	—	—	—	0	—	+	7.5	0.2	7.2	4.8	0.5	0.2	0.3	1.7	0.1	0	
kv	F <sub>28</sub>	—	+	—	0	—	+	5.8	0	3.8	5.7	5.0	0	0.2	0	0.1	0	
kw	F <sub>28</sub>	E	—	—	0	—	+	5.9	0	6.2	5.4	4.9	0.1	0	0	0.1	0	
ky	F <sub>29</sub>	—	—	—	0	—	+	5.2	0.1	5.7	5.4	5.1	3.8	5.9	0.1	0.1	0.1	
la	F <sub>30</sub>	—	—	—	0	—	+	5.5	0.1	4.8	5.0	4.6	5.9	6.2	0	0	0	
lb	F <sub>31</sub>	E	—	—	0	—	+	5.9	0	5.1	5.0	5.7	5.6	0	0.1	0	0	
lc	F <sub>31</sub>	—	—	—	0	—	+	6.0	0.1	5.4	4.7	0	0	0	0	0	0	
ld	F <sub>31</sub>	—	—	—	0	—	+	5.4	0	5.5	5.3	0	0	0	0	0.1	0.2	
le	F <sub>32</sub>	C	—	—	0	—	+	5.1	0	4.3	5.0	5.2	6.3	5.8	0	0	0	
lf	F <sub>33</sub>	—	—	—	36	—	+	4.9	0.1	5.8	5.2	4.6	0.2	0	0	0.2	0	
lg	F <sub>34</sub>	—	—	—	0	—	+	5.1	0.5	4.8	3.5	0.5	0.5	0.7	4.6	2.3	0.1	
lh	F <sub>35</sub>	G	—	—	0	—	+	6.2	0	6.5	6.0	4.5	6.1	6.4	4.5	0.1	0	
li	F <sub>35</sub>	C	—	+	0	—	+	6.6	0	6.4	1.7	4.7	0.1	0	4.3	0.1	0	
lj	F <sub>36</sub>	C	—	—	0	—	+	5.2	0	5.2	5.0	4.6	6.1	6.1	0	0.1	0	
lk	F <sub>36</sub>	H	—	+	0	—	+	6.3	0	4.9	4.7	4.6	0.2	0.1	0.2	0	0	
ll	F <sub>36</sub>	H	—	+	0	—	+	6.5	0	5.0	5.0	4.8	0	0	0.1	0	0	
lm	F <sub>37</sub>	D	—	—	0	—	+	5.7	0	5.0	4.6	4.4	6.0	6.4	0.1	0	0	
ln	F <sub>37</sub>	—	—	—	0	—	+	5.4	0.1	5.2	5.6	4.3	6.0	0.2	0	0	0	
lo	F <sub>37</sub>	E	—	—	0	—	+	6.1	0	4.9	5.1	4.9	5.9	6.3	0.1	0.1	0	
lp	F <sub>38</sub>	—	—	—	0	—	+	6.3	0	5.2	4.9	4.8	6.4	0	0	0	0	
lq	F <sub>39</sub>	E	—	+	0	—	+	6.6	0	6.3	5.2	4.8	0	0	0.2	0	0	
lr	F <sub>39</sub>	—	—	—	0	—	+	6.8	0	6.2	5.1	5.0	0	0	3.9	0	0	
ls	F <sub>39</sub>	E	—	+	0	—	+	6.5	0	6.4	4.7	4.4	0	0.2	0	0	0	
lt	F <sub>40</sub>	E	—	—	0	—	+	5.6	0	5.2	4.7	5.3	5.3	0.1	0.1	0	0	
lu	F <sub>40</sub>	G	—	—	0	—	+	5.8	0	5.3	5.6	6.0	6.2	0	0	0	0	
lv	F <sub>40</sub>	—	—	—	0	—	+	5.7	0	5.2	5.8	5.3	6.1	0.2	3.6	0	0	

TABLE II.—Physiological characters of all cultures—Continued.

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid.										
			Milk.	Broth.				Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.	
lx	F41	E	—	—	mm.	—	—	6.2	0	5.5	5.2	5.2	6.1	0.1	0	0	0.1	
ly	F41	E	—	—	0	—	—	6.8	0.1	5.3	5.1	5.0	6.3	0.1	0	0.2	0.1	
lz	F41	E	—	—	0	—	—	6.5	0.1	5.1	5.0	5.0	6.3	0	0	0.1	0.1	
ma	F42	E	—	—	0	—	—	6.2	0	4.3	5.4	5.4	6.2	0	0	0	0	
mc	F43	E	—	—	0	—	—	6.5	0	5.3	4.6	5.2	6.0	0	0	0	0	
md	F43	E	—	—	0	—	—	6.5	0	5.0	4.6	5.3	6.2	0	0	0	0	
me	F44	C	—	—	0	—	—	6.6	0	5.2	4.8	5.1	7.0	0	0	0	0	
mf	F45	C	—	—	0	—	—	6.6	0	5.3	5.0	5.5	4.6	0	0	0	0	
mg	F46	C	—	—	0	—	—	6.4	0	4.8	4.0	4.6	6.5	0	0	0	0	
mh	F46	E	—	—	0	—	—	5.4	0	5.4	4.6	4.3	0	0	0	0	0	
mi	F38	E	—	—	0	—	—	6.4	0	5.1	5.0	4.5	6.5	0	0	0	0	
mj	F38	E	—	—	0	—	—	6.3	0	5.1	4.6	4.9	0	0	0	0	0	
mk	F47	D	—	—	0	—	—	6.4	0	4.8	5.1	5.0	6.2	0	0	0	0	
ml	F47	D	—	—	0	—	—	6.0	0	5.0	4.9	4.9	6.1	0	0	0	0	
mm	F47	C	—	—	0	—	—	6.4	0	4.7	5.3	5.2	6.3	0	0	0	0	
mn	F48	E	—	—	0	—	—	5.6	0	5.9	5.5	5.1	0	0	0	0	0	
mp	F48	E	—	—	0	—	—	5.7	0	5.9	5.0	0	0	0	0	0	0	
mq	F49	E	—	—	0	—	—	6.2	0	5.3	5.3	5.5	6.2	0	0	0	0	
mr	F49	E	—	—	0	—	—	6.6	0	5.1	4.8	5.1	6.2	0	0	0	0	
ms	F50	E	—	—	0	—	—	6.4	0	5.1	4.9	0	6.1	0	0	0	0	
mt	F50	A	—	—	0	—	—	6.1	0	5.2	5.0	5.0	6.4	0	0	0	0	
mu	F51	A	—	—	0	—	—	6.0	0	5.1	5.0	5.0	6.4	0	0	0	0	
mv	F51	H	—	—	0	—	—	5.9	0.2	5.1	4.7	5.2	6.2	0	0	0	0	
mw	F52	E	—	—	0	—	—	5.7	0.1	5.0	4.8	5.4	6.2	0	0	0	0	
mx	F52	E	—	—	0	—	—	5.9	0.1	3.8	5.0	0	0	0	0	0	0	
my	F53	E	—	—	0	—	—	5.5	0.2	5.2	4.9	4.9	6.1	0	0	0	0	
mz	F53	E	—	—	0	—	—	5.8	0.1	3.9	4.8	0	0	0	0	0	0	
na	F54	E	—	—	0	—	—	5.0	0	5.0	5.2	5.4	5.3	0	0	0	0	
nb	F54	E	—	—	0	—	—	5.1	0	5.1	4.7	0	0	0	0	0	0	
nc	F54	E	—	—	0	—	—	4.4	0.2	5.1	4.9	0	0	0	0	0	0	
nd	F55	E	—	—	0	—	—	5.6	0	5.9	5.4	5.3	4.5	0	0	0	0	
ne	F55	E	—	—	0	—	—	5.9	0.2	6.0	5.4	5.5	0	0	0	0	0	
nf	F56	E	—	—	0	—	—	5.8	0.1	5.1	5.1	0	0	0	0	0	0	
ng	F56	E	—	—	0	—	—	4.7	0.1	5.2	5.5	0	0	0	0	0	0	
ni	U9	H	—	—	0	—	—	4.6	0	4.6	3.1	0	0	0	0	0	0	
nj	U9	H	—	—	0	—	—	4.9	0	5.2	5.0	0	0	0	0	0	0	
nk	U9	B	—	—	0	—	—	5.1	0.1	4.8	5.1	0	0	0	0	0	0	
nl	U10	C	—	—	35	—	—	7.2	0.1	4.7	5.1	0	0	0	0	0	0	
nm	U10	G	—	—	24	—	—	7.4	0	5.1	4.3	0	0	0	0	0	0	
nn	U10	H	—	—	32	—	—	7.4	0.2	4.4	5.1	0	0	0	0	0	0	
no	U11	H	—	—	0	—	—	4.4	0	4.7	3.8	0	0	0	0	0	0	
np	U12	H	—	—	0	—	—	5.8	0	0	4	0	0	0	0	0	0	
nq	U13	H	—	—	0	—	—	5.3	0	4.1	4.7	0	0	0	0	0	0	
nr	U14	B	—	—	0	—	—	6.4	0	5.6	1.5	0	0	0	0	0	0	
ns	U14	B	—	—	0	—	—	6.4	0	5.5	5.1	0	0	0	0	0	0	
nt	U15	H	—	—	0	—	—	5.8	0	5.1	4.8	0	0	0	0	0	0	
nu	U16	H	—	—	0	—	—	3.8	0	4.4	4.7	0	0	0	0	0	0	
nv	U16	H	—	—	0	—	—	5.9	0	4.7	4.8	0	0	0	0	0	0	
nw	U16	H	—	—	0	—	—	5.4	0.2	4.4	4.7	0	0	0	0	0	0	
nx	U17	H	—	—	0	—	—	5.9	0.1	4.0	4.5	0	0	0	0	0	0	
ny	U17	H	—	—	0	—	—	5.2	0.1	0	4.5	0	0	0	0	0	0	
nz	U17	H	—	—	0	—	—	6.0	0	4.5	4.6	0	0	0	0	0	0	
oa	B1	C	—	—	0	—	—	5.7	0.4	4.4	5.2	5.6	4.8	5.5	0	0	0	
ob	B1	C	—	—	0	—	—	4.4	0	5.3	4.9	4.4	4.1	0	0	0	0	
oc	B2	H	—	—	0	—	—	4.3	0	5.4	4.7	4.5	0	0	0	0	0	
od	B2	F	—	—	0	—	—	5.7	0	4.6	4.5	7.1	0	3.0	4.0	0	0	
oe	B3	F	—	—	0	—	—	5.4	0	4.9	5.0	4.1	0	3.3	3.8	0	0	
of	B3	F	—	—	0	—	—	5.8	0	4.5	4.6	4.0	0	3.3	3.9	0	0	
og	B4	F	—	—	0	—	—	5.4	0	4.0	5.1	3.9	0	3.0	3.8	0	0	
oh	B5	F	—	—	0	—	—	6.1	0	6.2	5.5	0	0	0	4.6	0	0	
oi	B6	E	—	—	0	—	—	5.9	0	5.8	5.0	1.7	0	3.7	3.9	0	0	
ol	B7	E	—	—	0	—	—	5.7	0.2	4.4	5.4	3.8	0	0	3.8	0	0	
om	B7	E	—	—	0	—	—	5.4	0	1.4	4.8	4.3	0	0	3.9	0	0	
on	B8	E	—	—	0	—	—	6.1	0.1	0	5.4	0	0	0	4.3	0	0	
oo	B8	E	—	—	0	—	—	6.4	0.1	0	5.5	0	0	0	4.2	0	0	
op	B9	E	—	—	0	—	—	6.0	0	0	5.3	0	0	0	4.5	0	0	
oq	B9	E	—	—	0	—	—	6.3	0.1	0	5.5	0	0	0	4.4	0	0	
or	B10	E	—	—	0	—	—	6.1	0	6.1	5.2	0	0	0	4.6	0	0	
os	B10	E	—	—	0	—	—	6.6	0	7.1	5.5	6.2	0	0	0	0	0	
ot	B11	E	—	—	0	—	—	6.0	0	6.0	5.4	0	0	0	4.4	0	0	
ou	B11	E	—	—	0	—	—	6.1	0	5.9	2.0	0	0	0	4.6	0	0	
ov	B12	E	—	—	0	—	—	6.9	0.1	5.9	5.1	0	0	0	0	0	0	
ow	B12	E	—	—	0	—	—	6.8	0.1	5.9	5.1	0	0	0	3.8	0	0	
ox	B13	E	—	—	0	—	—	6.3	0.1	5.9	5.1	0	0	0	4.0	0	0	

TABLE II.—*Physiological characters of all cultures—Continued.*

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red re- duction.	Milk curd.	Percentage of normal acid.										
			Milk.	Broth.				Dextrose.	Adonite.	Saccha- rose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.	
oy	B <sub>13</sub>	B	.....	—	mm.	—	—	6.6	0.1	5.8	5.6	0.1	0	0	3.7	0.2	0.1	
oz	U <sub>18</sub>	B	.....	+	0	—	++	4.9	0.1	4.4	4.5	0.1	0.1	0	0	0	0.2	
pa	U <sub>18</sub>	.....	.....	.....	0	—	++	5.2	0.1	4.1	4.3	0	0	0	0	0.1	0.1	
pb	U <sub>19</sub>	.....	.....	.....	0	—	++	4.9	0	4.6	5.3	0.2	0	0	0	0.2	0.2	
pc	U <sub>19</sub>	.....	.....	.....	0	—	++	5.0	0	5.2	5.3	0.1	0	0.2	0	0.1	0.1	
pd	B <sub>14</sub>	E	.....	—	0	—	++	4.8	0.2	6.2	4.5	0.1	0	0	3.9	0.2	0.1	
pe	B <sub>14</sub>	E	.....	—	0	—	++	4.3	0	4.1	4.6	0	0	0	3.5	0.1	0	
pf	B <sub>15</sub>	E	.....	—	0	—	++	5.3	0	4.4	5.0	3.8	0	2.8	3.7	0.2	0	
pg	B <sub>15</sub>	E	.....	—	0	—	++	5.4	0	4.5	5.0	3.9	0	2.9	3.6	0.3	0	
ph	B <sub>16</sub>	E	.....	—	0	—	++	6.0	0.2	1.8	4.5	0.1	0	0	4.3	0.1	0.2	
pi	B <sub>16</sub>	E	.....	—	0	—	++	5.9	0.3	1.8	4.7	0.1	0	0	4.7	0.2	0.1	
pj	B <sub>17</sub>	E	.....	—	0	—	++	4.7	0	4.1	4.7	0	0.1	0	3.5	0.1	0	
pk	B <sub>17</sub>	E	.....	—	0	—	++	4.6	0	4.2	4.8	0	0	0	3.9	0.2	0.3	
pl	B <sub>18</sub>	E	.....	—	0	—	++	5.5	0	4.2	5.0	3.9	0	2.8	3.7	0.5	0.1	
pm	B <sub>18</sub>	E	.....	—	0	—	++	5.6	0	4.2	4.8	4.0	0	2.8	3.8	0.4	0	
pn	B <sub>19</sub>	E	.....	—	0	—	++	4.8	0	4.1	4.6	3.3	0	0	3.6	0.2	0	
po	B <sub>19</sub>	E	.....	—	0	—	++	4.8	0	4.3	4.8	3.2	0	0	3.8	0.1	0.1	
pq	B <sub>20</sub>	E	.....	—	0	—	++	5.2	0	4.2	4.8	0	0.2	0.2	3.6	1.6	0.2	
pr	B <sub>20</sub>	E	.....	—	0	—	++	5.4	0.1	4.3	4.8	0	0	0.3	3.5	0.1	0.2	
ps	B <sub>21</sub>	E	.....	—	0	—	++	6.2	0.1	4.2	5.1	0	0	0.1	2.9	0	0	
pt	B <sub>21</sub>	E	.....	—	0	—	+	6.2	0	5.3	4.9	0	0	0.2	2.9	0	0	

In one particular our results do not agree with the conclusions reached by Stowell, Hilliard, and Schlesinger<sup>1</sup> and by Howe and others in that the "metabolic gradient" which they establish, in our opinion, can be correct only for the particular group under consideration, since the number of cultures utilizing any particular carbohydrates or similar compound is dependent on the peculiarities of the cultures as well as on the composition or the configuration of the test substance. While in a general way our cultures follow the scheme outlined by Stowell, Hilliard, and Schlesinger, this arrangement may be varied, as will be pointed out later, by varying the source from which the cultures are obtained. In one group of our collection a much larger percentage of cultures give a fermentation with mannite than with raffinose; in others the conditions are reversed. In no case did we obtain a higher percentage of positive results with mannite than with inulin, although both Winslow and Stowell, Hilliard, and Schlesinger put inulin above mannite. Dulcife may be considered as one of the more difficultly fermented alcohols, and yet in our work on the colon group we found that dulcife was fermented most frequently, not by the more active group but by the one which otherwise showed weak fermentative ability. With adonite the conditions were reversed.

There is among all acid-forming bacteria and especially among the streptococci considerable variation in the maximum amount of acid produced. Winslow has shown that this may be a valuable aid in dis-

<sup>1</sup> Stowell, E. C., Hilliard, C. M., and Schlesinger, M. J. A statistical study of the streptococci from milk and from the throat. Jour. Infect. Diseases, v. 12, no. 2, p. 144-164. 1913.

tinguishing cocci of different species.<sup>1</sup> Stowell, Hilliard, and Schlesinger, in the paper already quoted,<sup>2</sup> have pointed out the marked difference in this regard between streptococci from milk and those from the human throat. In Table III is shown the distribution of cultures according to their source and the quantity of acid formed in dextrose broth. This is also shown graphically in figure 4. The mode for the culture from the mouth falls over 6.5 per cent, while that for the udder organisms is over 5.0 per cent, and that for those from feces is 5.5 per cent. The mode for each group is sharply defined, especially those for the udder and feces groups. On the assumption that the cultures obtained from milk may have come originally from any of the other sources, we would expect the curve representing the milk cultures to spread over the space occupied

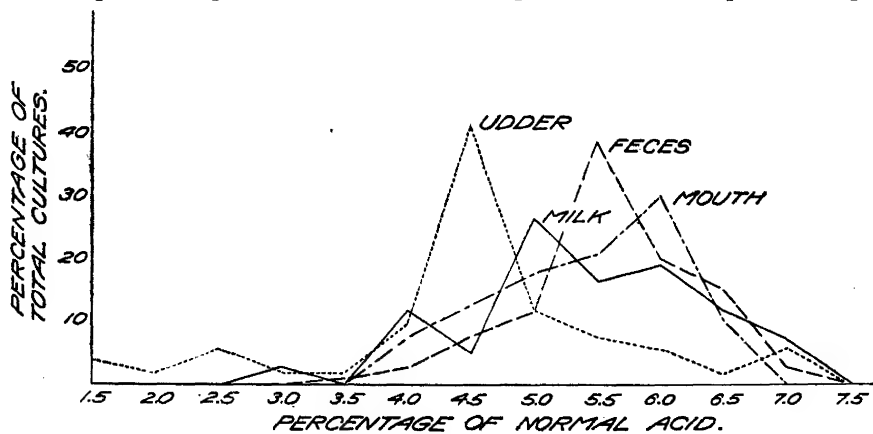


FIG. 4.—Frequency curves showing acid formation in dextrose broth.

by the other curves. This is true in a general way, but the curve for the milk cultures has a mode falling between that for the udder and the feces cultures. It should be remembered that the milk cultures were not selected promiscuously but from bile tubes incubated at 37° C.

TABLE III.—Distribution of cultures according to the percentage of normal acid produced in dextrose broth.

Source.	Total number of cultures.	Below 1.0.	1 to 1.5.	1.5 to 2.0.	2.0 to 2.5.	2.5 to 3.0.	3.0 to 3.5.	3.5 to 4.0.	4.0 to 4.5.	4.5 to 5.0.	5.0 to 5.5.	5.5 to 6.0.	6.0 to 6.5.	6.5 to 7.0.	7.0 to 7.5.	Above 7.5.
Milk:																
Number	42	0	0	0	0	0	1	0	5	2	11	7	8	5	3	0
Per cent.		0	0	0	0	0	2.38	0	11.90	4.76	26.19	16.67	19.05	11.90	7.14	0
Udder:																
Number	51	0	0	2	1	3	1	1	5	21	6	4	3	1	3	0
Per cent.		0	0	3.92	1.96	5.88	1.96	1.96	9.80	41.18	11.76	7.84	5.88	1.96	5.88	0
Feces:																
Number	114	0	0	0	0	0	0	1	3	9	13	44	23	18	3	0
Per cent.		0	0	0	0	0	0	0.88	2.63	7.89	11.40	38.59	20.17	15.79	2.63	0
Mouth:																
Number	39	0	0	0	0	0	0	0	3	5	7	8	12	4	0	0
Per cent.		0	0	0	0	0	0	0	7.69	12.82	17.95	20.51	30.77	10.26	0	0

<sup>1</sup> Winslow, C. E. A., and Winslow, Anne R. Systematic relationships of the Coccaceae. ed. 1, 300 p., illus. New York, 1908.

<sup>2</sup> Stowell, Hilliard, and Schlesinger. Op. cit.

ACTION ON LITMUS MILK

Late in the course of the investigation it was noticed that there were distinct differences in the action of different cultures on the litmus in milk and that this difference was in some relation to the source of the cultures. Some cultures decolorized the litmus promptly, leaving a white curd, with the exception of a pink ring at the top, which slowly extended downward. Other cultures produced a curd which remained pink throughout for an indefinite period. This action was recorded for the cultures then available, and the results are given in Table IV. It will be noticed that while the ability to reduce litmus is characteristic of the mouth cultures it is almost entirely lacking in the cultures from

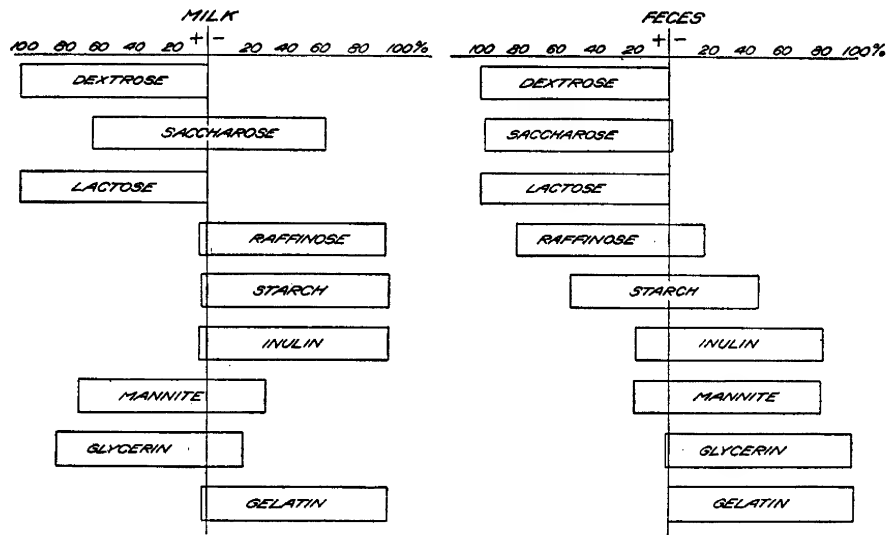


FIG. 5.—Graphic representation of the characters of cultures of streptococci from milk and from bovine feces.

the udder. The number of cultures in the two other groups in which this character was recorded is too small to permit conclusions, but there may be observed a tendency in the milk cultures to agree with those from the udder.

TABLE IV.—Distribution of cultures according to action on litmus in milk.

Cultures recorded from—	Number of cultures.	Cultures reducing litmus.		Cultures failing to reduce litmus.
		Number.	Percent.	
Milk.....	17	4	23.53	Per cent. 76.57
Feces.....	16	6	37.50	62.50
Udder.....	29	2	6.89	93.10
Mouth.....	35	29	82.86	17.14



## THE FERMENTATION OF TEST SUBSTANCES

In Table V the cultures are arranged on the basis of fermentation or nonfermentation of eight test substances. In this table all reactions of 1 per cent or over are counted as positive and those falling below as negative. The results given in this table are arranged in a form more easily studied in figures 5 and 6. In these diagrams all positive results are plotted to the left of a vertical line and the negative results to the right. The udder organisms are characterized by the general lack of ability to ferment the test substances. They fail almost without exception to ferment raffinose and the polysaccharids, but show some tendency to attack the two alcohols. On the other hand, the 114 cultures

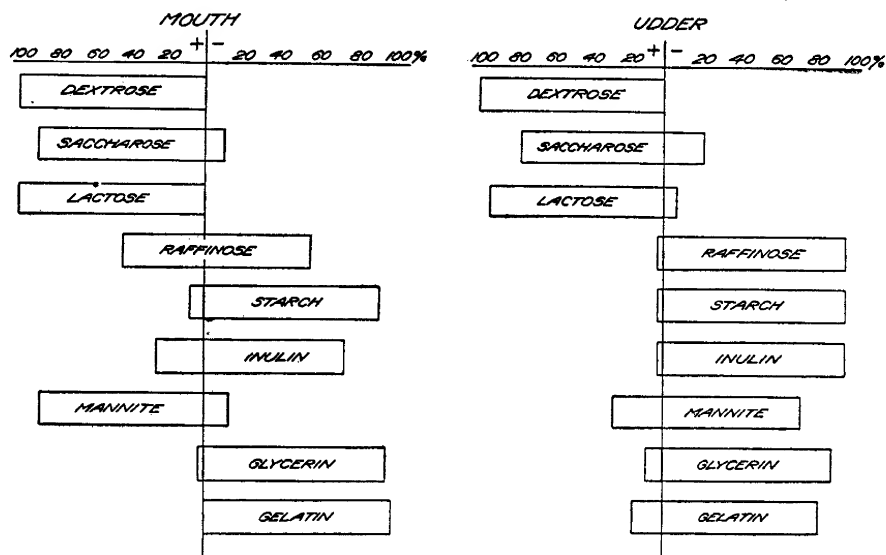


FIG. 6.—Graphic representation of the characters of cultures of streptococci from the mouths of cows and from infected udders.

from bovine feces fail almost entirely to utilize the alcohols, but show exceptional activity in fermenting the more complex sugars and the polysaccharids.

TABLE V.—Fermentation of test substances.

Origin of culture.	Dextrose.		Saccharose.		Lactose.		Raffinose.		Starch.		Inulin.		Mannite.		Glycerin.	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
<b>Milk:</b>																
Total.....	42	0	21	21	42	0	2	40	1	41	2	40	29	13	34	8
Percentage of total....	100	0	50	50	100	0	4.8	95.2	2.3	97.7	4.8	95.2	69.0	31.0	80.9	19.1
<b>Udder:</b>																
Total.....	51	0	40	11	48	3	0	51	2	49	2	49	14	37	6	45
Percentage of total....	100	0	78.4	21.6	94.3	5.7	0	100	4	96	4	96	27.4	72.6	11.6	88.4
<b>Feces:</b>																
Total.....	114	0	112	2	114	0	93	21	60	54	20	94	21	93	2	112
Percentage of total....	100	0	98.2	1.8	100	0	81.5	18.5	52.5	47.5	17.6	82.4	18.5	81.5	1.8	98.2
<b>Mouth:</b>																
Total.....	40	0	35	4	39	0	17	22	3	36	10	29	34	5	1	38
Percentage of total....	100	0	89.7	11.3	100	0	43.6	56.4	7.7	92.3	25.6	74.4	87.2	12.8	2.6	97.4

The cultures from the mouth differ from those from the udder in the higher percentages of raffinose, inulin, and mannite fermenters and in less action on glycerin and gelatin. They are sharply differentiated from the feces organisms in their general failure to ferment starch and the much higher percentage of mannite fermenters.<sup>1</sup>

The milk cultures are distinguished by the comparatively small number of saccharose fermenters, the failure to ferment raffinose, starch, and inulin, and the active fermentation of both mannite and glycerin.

#### THE LIQUEFYING CULTURES

It will be noted that with the exception of a few obtained from milk, all of the liquefying cultures came from the udder. If we consider the 11 gelatin-liquefying cultures as a group we obtain the data given in Table VI, which shows that the liquefaction of gelatin is not an isolated variation from the type but is correlated with an ability to utilize the alcohols, mannite, and glycerin. This peculiar correlation between gelatin liquefaction and glycerin fermentation was also noticed in the colon group.

TABLE VI.—*Comparison of liquefying and nonliquefying cultures of streptococci from the udder.*

Item.	Gela- tin.	Dex- trose.		Sac- charose.		Lactose.		Raffi- nose.		Starch.		Inulin.		Mannite.		Glycerin.	
		+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Number of cultures . . . . .	+	11	0	10	1	11	0	0	11	1	10	0	11	9	2	6	5
Per cent. . . . .		100.0	0	90.90	9.09	100.0	0	0	100.0	9.09	90.90	0	100.0	81.81	18.19	54.54	45.46
Number of cultures . . . . .	-	43	0	33	10	40	3	0	43	2	41	2	41	7	36	0	38
Per cent. . . . .		100.0	0	76.74	23.26	93.02	6.98	0	100.0	4.65	95.35	4.65	95.35	16.28	83.72	0	100.0

The characters of the 11 cultures included in Table VI agree very closely with the 'Group C' of the article by the writers on the lactic-acid bacteria.<sup>2</sup> If we divide the udder cultures into gelatin-liquefying and nonliquefying groups, we obtain figure 7, in which the cultures are arranged as in figures 5 and 6. This gives two groups in each of which the cultures show distinctive characters and remarkable uniformity.

We have, then, at least three sharply defined varieties: Two from the udder, of which one has weak fermentative ability, attacking only dextrose, saccharose, and lactose, with an occasional culture-producing acid from mannite, inulin, or starch, and a second less numerous type, which liquefies gelatin and ferments dextrose, saccharose, lactose, mannite, and usually glycerin; and one from bovine feces, character-

<sup>1</sup> Since this paper was written, a communication by C. A. Fuller and V. A. Armstrong entitled "The differentiation of fecal streptococci by their fermentative reactions in carbohydrate media" has appeared in the Jour. of Infect. Diseases, v. 13, no. 3, p. 442-462, Nov., 1913. The characteristics of their cultures from bovine feces agree in all essential particulars with those found by the writers.

<sup>2</sup> Rogers, L. A., and Davis, B. J. Methods of classifying the lactic-acid bacteria. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 154, 30 p., 6 fig. 1912.



The same test applied to the mouth cultures would show that almost any individual culture could be included in the feces group. However, almost any mouth culture would be an exceptional, not a typical, feces culture. A culture fermenting saccharose, lactose, raffinose, and mannite could be either from the mouth or from feces, but there is a high probability that it would be of buccal origin. On the other hand, a culture fermenting saccharose, lactose, raffinose, and starch, but failing to ferment mannite or glycerin, would almost certainly be of fecal origin.

#### RELATION OF THESE GROUPS TO NAMED VARIETIES

It would be difficult to identify all of these groups with previously described species. Until the work of Gordon, few cultures were described

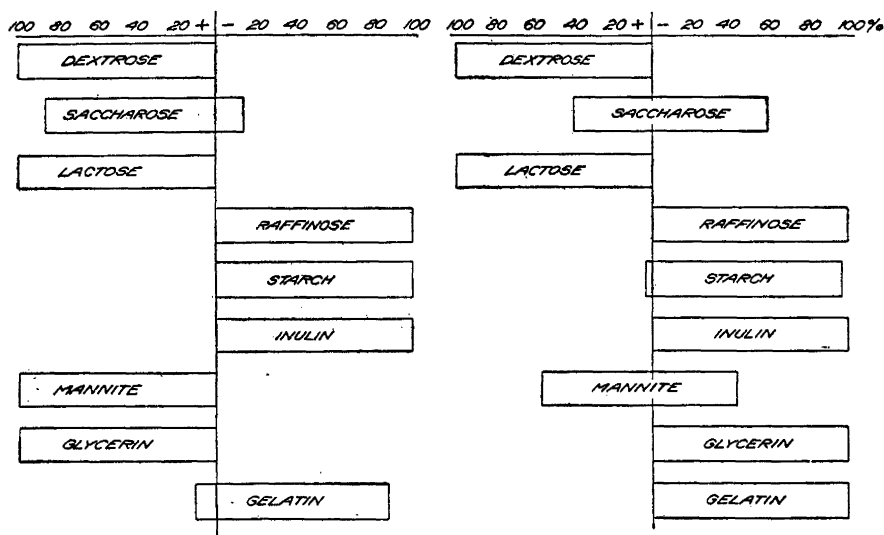


FIG. 8.—Diagram showing a possible grouping of the milk cultures of streptococci.

on the basis of the fermentation of a large number of test substances, and in only a very few cases have the cultures been obtained from a definite source. An exception may be made of the pathogenic bacteria in which the cultures described have been selected from definite and very similar sources. Among the streptococci we have an example in the pus-forming organism generally described as *Streptococcus pyogenes*. In Table VIII are compiled the typical reactions given for *Streptococcus pyogenes* by three investigations. The reactions given by Andrewes and Horder are compiled from a large number of cultures, and those given by Gordon are from a number of his own cultures.<sup>1</sup> Those given by Bergey are the reactions of a comparatively few typical cultures.<sup>2</sup> So

<sup>1</sup> Andrewes, F. W., and Horder, T. J., A study of the streptococci pathogenic for man. *Lancet*, v. 2, no. 11, p. 708-713; no. 12, p. 775-782; no. 13, p. 852-855. 1906.

Gordon, M. H. Report on an investigation of the fermentative characters of streptococci present on fauces during scarlet fever. 40th Ann. Rpt. Local Govt. Bd. [Gt. Brit.], 1910-11, Suppl. Rpt. Med. Off., p. 302-31, 1911.

<sup>2</sup> Bergey, D. H. Differentiation of cultures of streptococcus. *Jour. Med. Research*, v. 27 (n. s., v. 12), no. 1, p. 67-77. 1912.

far as it is possible to make comparisons, the reactions given agree very closely with our nonliquefying udder cultures.

TABLE VIII.—Results of fermentation tests of *Streptococcus pyogenes* described in the literature.

Authority.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Salicin.	Coniferin.
Our nonliquefying udder cultures... per cent..	76	93	0	4	4	16	16	.....	.....
Andrewes and Horder.....	+	+	+	.....	+	+	.....	+	+
Gordon.....	+	+	+	.....	+	+	.....	+	+
Bergey.....	+	+	+	.....	+	+	.....	+	+

A still further comparison is possible by the tabulation of the fermentation reactions of five typical cultures of *Streptococcus pyogenes* obtained through the courtesy of Prof. C. E. A. Winslow, of the American Museum of Natural History. These results are given in Table IX. Although some of these cultures have been grown on artificial media for many years, they still exhibit the same general characters as our freshly isolated udder cultures—namely, an ability to ferment dextrose, saccharose, and lactose, general failure to ferment raffinose and the polysaccharids, and an erratic tendency to ferment the alcohols. Unfortunately the gelatin test was not made on these five cultures. The fermentation of glycerin by three of the five indicates that they may have been of the liquefying type. Savage in 176 cultures of streptococci isolated from cases of mastitis found that 95 per cent liquefied gelatin.<sup>1</sup> His cultures differed from both the typical *S. pyogenes* and our liquefying cultures in that 49 per cent fermented raffinose.

TABLE IX.—Results of fermentation tests of five cultures of *Streptococcus pyogenes* from American Museum of Natural History (New York) collection.

Source.	Dextrose.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.
New York Post Graduate Medical College (fatal septicemia).....	3.90	3.85	3.60	0.20	0.18	0.23	0.35	0.59
Dr. Bien, Chicago, Ill. (abscess in erysipelas).....	5.45	4.75	3.25	0	.13	.18	2.55	2.34
Boston Board of Health (urine).....	3.85	4.05	.45	0	3.98	0	0	1.74
Johns Hopkins University.....	6.45	4.95	4.70	.20	.45	.08	4.91	1.67
Michigan Agricultural College.....	2.50	0	1.15	.05	0	.09	.23	.19

#### VARIATION FROM TYPE IN THE UDDER ORGANISMS

The trouble from infected udders at both the Beltsville and Annapolis farms was in the nature of an epidemic. The infection apparently spread from cow to cow and became so severe that at Annapolis one or

<sup>1</sup> Savage, W. G., Report upon the bacteriology and pathology of "Garget" (or mastitis) in cows. 37th Ann. Rept. Local Govt. Bd. [Gt. Brit.], 1907-8. Suppl. Rept. Med. Off., pp. 359-424. 1909.

more cows were rendered useless. There was no apparent connection between the two epidemics except that they occurred at about the same time. We may assume that these epidemics originated in one of two ways, either of which must admit more or less variation in physiological reactions from the original type. It may be possible that the udders of one or more cows may have become infected by some of the streptococci coming originally from the mouth, intestines, or other sources. Under the influence of its new environment this organism may have acquired pathogenic properties sufficient to produce the symptoms observed in mammitis. Heinemann has shown that pathogenicity is a property readily acquired when ordinary streptococci are grown in animals.<sup>1</sup> If these infecting organisms came from the mouth, the intestines, or the milk they must have acquired in a comparatively short time an entirely new set of biochemical reactions in addition to a variation in pathogenicity. On the other hand, we may assume with much more appearance of reasonableness that the infection spread from a single infecting cell or aggregate of similar cells which already possessed pathogenic powers and general characters identical with those we have found to be characteristic of the udder organisms. This assumption is in accord with the established fact that streptococci from pathological lesions in general have similar biochemical reactions. If the infection in these two cases came from various sources, it must follow that growth under similar conditions would produce uniform fermentation reactions in a short time, a view held by Walker, who maintains that these reactions may be varied almost at will and can only indicate the latest habitat of the culture.<sup>2</sup> If the infection came from a single cell, there must have been some variation, since the fermentation reactions were not identical at the time of this isolation.

In Table X are shown the varieties of nonliquefying udder cultures and the number occurring in each of the two herds. There were seven varieties in all. The most numerous one ferments dextrose, saccharose, and lactose only and occurred 24 times, equally divided between the two herds. The next most numerous variation differed from the first in failing to ferment saccharose and occurred 8 times. A third variation fermented mannite in addition to dextrose, saccharose, and lactose and occurred 4 times. The remaining four varieties evidently occur only once or twice in every 40 cultures. Viewed from any standpoint it is evident that these organisms are subject to variation from the type, but, these variations are not of sufficient magnitude or frequency to detract from the value of the physiological reactions as a means of establishing true species.

<sup>1</sup> Heinemann, P. G., The pathogenicity of *Streptococcus lacticus*. Jour. Infect. Diseases, v. 4, no. 1, p. 87-92. 1907.

<sup>2</sup> Walker, E. W. A., On variation and adaptation in bacteria, illustrated by observations upon streptococci, with special reference to the value of fermentation tests as applied to these organisms. Proc. Roy. Soc. [London], s. B, v. 83, no. 567, p. 541-558. 1911.

TABLE X.—*Variation from type in nonliquefying udder cultures.*

Significant characters.								Number of cultures from herd at—		Total number of cultures.
Dextrose.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Beltsville.	Annapolis.	
+	+	+	—	—	—	—	—	12	12	24
++	++	++	—	—	—	—	—	6	2	8
+++	+++	+++	—	—	—	—	—	2	2	4
++++	++++	++++	—	—	+	+	—	1	1	2
++++	++++	+	—	—	+	+	—	0	1	1
++++	+	+	—	—	+	+	+	1	0	1
++++	+	+	—	—	+	+	+	1	0	1

## SUMMARY

A collection of cultures of streptococci was made consisting of 42 cultures from milk which formed chains in lactose bile at 37° C., 51 cultures from infected udders, 114 cultures from bovine feces, and 39 cultures from the mouths of animals.

The morphology varied under different conditions and could not be correlated with the source of the culture, except that the udder cultures had a more marked tendency to chain formation than those from other sources.

The ability of these cultures to liquefy gelatin and to form acid from dextrose, lactose, saccharose, raffinose, starch, inulin, mannite, glycerin, dulcitol, and adonitol was determined. Only one or two cultures utilized adonitol or dulcitol.

When glycerin was attacked, the fermentation proceeded slowly, failing to reach its maximum in 14 days, in contrast to the fermentation of the sugars, in which the maximum was reached in two or three days.

A high percentage of the udder cultures failed to give the characteristic reduction in litmus milk.

Twelve cultures liquefied gelatin; one of these came from milk and 11 from infected udders.

The cultures from feces were characterized by their activity in fermenting the sugars, including raffinose, and their inability to utilize the alcohols.

The mouth cultures fermented dextrose, saccharose, lactose, mannite, and frequently raffinose, but were almost without effect on starch and glycerin.

The udder cultures were characterized by the general lack of fermentative ability, which was limited almost entirely to dextrose, saccharose, and lactose, with a comparatively small number utilizing mannite, glycerin, and gelatin.

When the udder cultures were divided on the basis of gelatin liquefaction, two groups were obtained. The fermentative activities of one

of these, which are similar to those of *Streptococcus pyogenes*, were limited to dextrose, saccharose, and lactose, with an occasional culture fermenting mannite, starch, or inulin. The second group fermented the three simple sugars, mannite, and usually glycerin and liquefied gelatin.

When the milk cultures were considered individually, it was found that with the exception of two which clearly came from feces they could be included in one or the other of the two groups into which the udder cultures were divided.

Of the 41 nonliquefying udder cultures 24 gave identical reactions. The remaining cultures differed from the type in one or two characters only.

28736°—14—6





# PRELIMINARY AND MINOR PAPERS

## CRYSTALLIZATION OF CREAM OF TARTAR IN THE FRUIT OF GRAPES

By WILLIAM B. ALWOOD,

*Chief, Enological Laboratory, Bureau of Chemistry*

During the chemical examinations made of the ripening fruit of grapes in the Enological Laboratory, Charlottesville, Va., the writer was led to conclude that the acid salt bitartrate of potassium was deposited from the juice in quantity sufficient to sensibly affect the analytical results. This led to the preparation of samples by the complete exhaustion of the soluble constituents of the berries, with results which supported the above conclusion.

The question of the character and location of the crystals of cream of tartar in the berry presented itself as a matter of interest and possibly of practical importance. The literature available did not furnish specific information on this point. Babo and Mach, in their exhaustive treatise, give but one brief reference to the occurrence of this salt in crystals in the fruit.<sup>1</sup>

As soon as the fruit was well colored at Charlottesville in 1912 a series of microscopic examinations was undertaken to determine whether crystals of bitartrate of potassium occurred in the fruit. Portions of Concord grapes were prepared and examined daily until the fruit was ripe. Minute crystals varying much in shape and size were found in great abundance in the soft cells lying just beneath the skin of the fruit. Crystals were not present at any time in the pulp or compact portion of the flesh in which the seeds are contained. Like examinations of Concord and Catawba were carried on at Sandusky, Ohio, in September and October, 1912, and crystals of the same general type were found.

The fact that many of the crystals found in the berries did not conform in type to crystals of the bitartrate prepared from pure cream of tartar made it doubtful as to whether potassium bitartrate was deposited or not. Therefore, the fruit was separated into portions for the purpose of a chemical examination covering this point. The tough pulp containing the seeds of 1,500 grams of ripe berries was separated from the hulls and soft peripheral layer of cells which adhere to the hulls. This layer contains the coloring matter. The hulls and pulp were then carefully pressed by hand and the juice of each recovered and held separately. This gave three portions: (1) The pressed hulls, (2) the juice recovered from the hulls, and (3) the juice recovered from the pulp.

In preparing the sample all the juice possible was recovered from the sample of hulls and pulp; that is, they were entirely exhausted so far as crushing and pressing could accomplish this result. The pressed hulls were then carefully macerated in distilled water until the soluble organic matter was exhausted. These portions showed on analysis the results given in Table I.

<sup>1</sup> Babo, A. F., and Mach, E. *Handbuch des Weinbaues und der Kellerwirtschaft*. Aufl. 4, Bd. 2, p. 16. Berlin, 1910.

TABLE I.—Analyses of Concord grapes in 1912, giving the percentage by weight of acids and acid salts.

Portion analyzed.	Total acid.	Total tartaric acid.	Free tartaric acid.	Cream of tartar.
Hulls exhausted with water.....	0. 429	<sup>a</sup> 0. 589	0. 08	0. 56
Juice pressed from hulls.....	. 141	. 054	. 00	. 07
Juice pressed from pulp.....	1. 065	. 724	. 20	. 59

<sup>a</sup> The results show for the samples of "hulls" a greater content of tartaric acid than the total titratable acid of the samples. This is always the case in grape samples where the "acids other than tartaric" fall below a certain proportion.

The results show that the juice pressed from the hulls is very low in acid and acid salts, and that, while the organic matter remaining in the hulls after pressure is less than half as acid as the pulp, it is rich in tartaric acid and cream of tartar, in these regards nearly equaling the percentage found in the juicy pulp. The actual weight of the pressed hulls was 304 grams, or one-fifth of the original sample of fruit. From the results given, it would appear that the hulls when pressed dry still retained the crystals observed with the microscope, and actual observation has demonstrated this fact. The results for tartaric acid and cream of tartar settle the point as to the composition of these crystals.

Analyses of like import were made at Sandusky, Ohio, of samples of Catawba and Concord grapes. The results show that the acid content of the soft layer of cells attached to the hulls is proportionally richer in tartaric acid and cream of tartar than the pulp.

In 1913 the microscopic examinations were begun much earlier, and four varieties of grapes were included—Delaware, Concord, Niagara, and Norton. The presence of crystals of bitartrate of potassium could be observed before the berries were all colored, and the analyses of partly ripe fruit confirm the results of 1912. These samples were separated into two portions only, the hulls and the pulp, as noted above; then each sample was completely exhausted of soluble organic matter by repeated macerations and heating in distilled water. Table II gives the results for one set of samples from each of two varieties.

TABLE II.—Analyses of grapes in 1913, giving percentage by weight of acids and acid salts

## CONCORD.

Portion analyzed.	Total acid.	Total tartaric acid.	Free tartaric acid.	Cream of tartar.
Hulls.....	0. 95	<sup>a</sup> 1. 11	0	1. 33
Pulp.....	1. 43	. 79	. 04	. 82

## NIAGARA.

Portion analyzed.	Total acid.	Total tartaric acid.	Free tartaric acid.	Cream of tartar.
Hulls.....	0. 67	<sup>a</sup> 0. 93	0	1. 18
Pulp.....	. 96	. 83	. 18	. 57

<sup>a</sup> The results show for the samples of "hulls" a greater content of tartaric acid than the total titratable acid of the samples. This is always the case in grape samples where the "acids other than tartaric" fall below a certain proportion.

There are crystals other than bitartrate present in the fruit, but this paper is intended only to record an observation which may have peculiar interest. Further details of the investigation will appear later.

# THE REDUCTION OF ARSENIC ACID TO ARSENIOUS ACID BY THIOSULPHURIC ACID

By ROBERT M. CHAPIN,  
*Senior Biochemist, Bureau of Animal Industry*

While endeavoring to work out a practicable field method for the estimation of the total arsenic—that is, a method which should include both arsenites and arsenates—in arsenical baths used for dipping cattle, studies were made upon the effect of various reducing agents which are able to absorb iodine in acid solution upon the well-known reversible reaction,  $\text{As}(\text{OH})_3 + 2\text{I} + 2\text{H}_2\text{O} \rightleftharpoons \text{As}(\text{OH})_5 + 2\text{HI}$ . Unless the solution in which this reaction is taking place is freely acidified with a strong mineral acid or heated, the progress of the reaction from right to left is inconveniently slow. It was found that the addition of sodium thiosulphate apparently so greatly aided the reduction that it rapidly went to completion, even in cold and but slightly acid solutions. From this observation it was but one step to discover that the presence of hydriodic acid played no part whatever, the reduction of arsenic acid to arsenious acid being quickly and completely effected by treatment with a mixture of sodium thiosulphate and mineral acid alone.

It has long been known that arsenic, like some other metals, may be quantitatively precipitated as sulphid by sodium thiosulphate in a boiling acid solution. In the present case, however, provided the conditions are right, there is no formation of arsenious sulphid.

The reactions which may follow from the acidification of a solution of sodium thiosulphate are complex and variable, depending upon temperature, dilution, relative proportions of thiosulphate and acid, and possibly upon the order in which the admixture is made. The matter has most recently been discussed by Stiasny and Das,<sup>1</sup> who studied the reactions between such a mixture and potassium bichromate, a problem similar in nature to the one here under consideration.

Preliminary experiments showed that (1) the rapidity with which the reduction of arsenic acid progresses is mainly dependent upon the concentration of hydrogen ions, the organic acids, except oxalic, operating very sluggishly, and (2) the nature of the reactions probably depends to a considerable extent upon whether arsenic or thiosulphuric acid is in excess and is also varied by the order in which the three components, arsenic acid, thiosulphate, and mineral acid, are mixed if the operation of mixing occupies any considerable time.

The present series of experiments was limited to the study of the reactions occurring when a mixture of arsenic acid, or arsenate, with excess of sodium thiosulphate is acidified with an appropriate amount of hydrochloric or sulphuric acid, such being the conditions which must necessarily prevail in any method for the quantitative estimation of arsenic which might be based on the reactions. The solutions employed were

<sup>1</sup> Stiasny, Edmund, and Das, B. M. Reaction between sodium thiosulphate and a mixture of potassium bichromate and sulphuric acid. A contribution to the chemistry of chrome tannage. *Jour. Soc. Chem. Indus.*, v. 31, no. 16, pp. 753-759. 1912.

(1) a tenth-normal (oxidimetric) solution of arsenic acid prepared by oxidizing arsenious acid with nitric acid and expelling excess of the latter, (2) a tenth-normal solution of sodium thiosulphate, (3) a twentieth-normal solution of iodine, free from iodate, and (4) normal hydrochloric acid. The equivalents of the solutions were as follows:

Ten c. c. of the solution of arsenic acid reduced, after Williamson, with hydrochloric acid and potassium iodide and then rendered alkaline with an excess of sodium bicarbonate required 19.74 c. c. of the iodine solution.

Twenty c. c. of the solution of sodium thiosulphate required 39.50 c. c. of the iodine solution. To the solution of sodium tetrathionate thereby resulting there were added 10 grams of dry sodium carbonate and the solution, loosely covered, was heated one hour upon a steam bath. It was then cooled, diluted, acidified to litmus paper with acetic acid, and without delay titrated with iodine solution, of which 39.45 c. c. were required.

In the experiments to be described a measured quantity of arsenic acid was diluted to 25 c. c. and was mixed—whether previously neutralized or not appeared to be immaterial—with 20 c. c. of thiosulphate added from a burette, and then with 10 c. c. of normal hydrochloric acid added from a pipette. When containing moderate amounts of arsenic, the mixtures remained perfectly clear for possibly 15 minutes, disengaging but a slight odor of sulphur dioxide. After a variable time an opalescence would appear, rapidly increasing and becoming yellow and accompanied by a pronounced odor of sulphur dioxide. For quantitative work the action obviously must be stopped before the separation of sulphur and arsenious sulphide becomes perceptible. From the considerable number of experiments only enough will be described to show the nature of the reactions occurring.

**EXPERIMENT NO. 1.**—Ten c. c. of the solution of arsenic acid, 15 c. c. of water, 20 c. c. of the solution of sodium thiosulphate, and 10 c. c. of hydrochloric acid were mixed and allowed to stand for  $7\frac{1}{2}$  minutes. The solution was then titrated with the iodine solution, using starch indicator (titration I), after which sodium bicarbonate was added, avoiding unnecessary excess, and titration with iodine continued (titration II). The end point of titration II was but briefly persistent, owing to the tendency of sodium tetrathionate to be oxidized to sulphate by iodine in alkaline solution. Next, 10 grams of dry sodium carbonate were added and the solution, loosely covered, was heated for 1 hour on a steam bath. Then it was cooled, somewhat diluted, acidified to litmus paper by acetic acid, and immediately titrated again with iodine (titration III). The results obtained were as follows:

Titration I.....	20.50 c. c. of iodine.
Titration II.....	19.75 c. c. of iodine.
Titration III.....	35.25 c. c. of iodine.

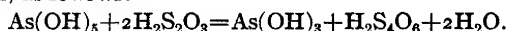
**EXPERIMENT NO. 2.**—Experiment No. 1 was duplicated, with the single exception that the mixture was allowed to stand but  $2\frac{1}{2}$  minutes before titration I was started. The results were as follows:

Titration I.....	20.55 c. c. of iodine.
Titration II.....	19.80 c. c. of iodine.
Titration III.....	35.55 c. c. of iodine.

Titration I removes the excess of reducing agent without affecting any arsenious acid present, provided a sufficient quantity of hydriodic acid be also contained in the solution. To insure this condition, it is safer to add a little potassium iodide just before beginning titration I, though in case of experiments Nos. 1 and 2 sufficient was introduced during the titration itself. Titration II measures the arsenious acid formed by the reduction of arsenic acid.

Comparing now the results of titration II with the iodine equivalent of the arsenic-acid solution, it is evident that the reaction is quantitative as respects arsenic. Comparing the sums of titrations I and II (1) 40.25 and (2) 40.35 c. c.) with the iodine equivalent of the thiosulphate solution (39.50 c. c.), it appears that the formation of

sulphurous acid is very slight and that the essential reaction involves the formation of tetrathionic acid, as follows:



A notable formation of any other acids of sulphur would necessarily result in a markedly higher figure for titration I.

Corroborative evidence of the essential transformation of thiosulphuric acid to tetrathionic acid is given by titration III, for Stiasny and Das have shown that an alkali tetrathionate, heated with sodium or potassium carbonate, is nearly quantitatively reconverted to thiosulphate. Titration III shows reformation of a quantity of thiosulphate equivalent to (1) 35.25 c. c. and (2) 35.55 c. c. of iodine solution, compared with an originally introduced quantity of thiosulphate equivalent to 39.50 c. c. of iodine, which amount of thiosulphate, as already noted, after oxidation to tetrathionate, digestion with sodium carbonate, and repeated titration, required 39.45 c. c. of iodine solution.

To further prove the actual reduction of arsenic acid and also that such reduction is brought about by thiosulphuric acid in the absence of hydriodic acid, the theoretically possible action of which is not rigorously excluded by the conditions of experiments Nos. 1 and 2, the following experiment was performed:

EXPERIMENT NO. 3.—A mixture of arsenic acid, sodium thiosulphate, and hydrochloric acid, made exactly as described in experiments Nos. 1 and 2, was allowed to stand for five minutes. After the addition of methyl orange, normal caustic soda was run in until only faint acidity remained, as shown by the orange tint of the solution. After the addition of a little sodium acetate and a drop or two of acetic acid to insure a distinctly acid reaction to litmus paper the solution was titrated cold with uranium acetate, using potassium ferrocyanide as indicator. The end point was reached upon the addition of 1 c. c. of uranium-acetate solution. Five c. c. of the arsenic-acid solution was then added and titration continued. The end point was again reached upon the addition of 10 c. c. more of uranium acetate, or a total of 11 c. c. Lastly, 5 c. c. of the arsenic-acid solution, treated in a parallel manner, but without any addition of sodium thiosulphate, required 10.75 c. c. of uranium-acetate solution. The previous conclusions regarding the nature and extent of the action upon arsenic acid were therefore confirmed.

As previously indicated, a small amount of the thiosulphuric acid suffers decomposition into sulphur dioxide and, presumably, sulphur. The sulphur does not become evident under the conditions observed, being partly held in colloidal solution, but for the most part reacting with tetrathionic acid to form pentathionic acid, as shown by Stiasny and Das in their investigations already mentioned. The presence of pentathionic acid was here shown in a similar manner on several of the mixtures while they still remained clear by neutralizing with caustic alkali, using methyl orange as indicator. As the neutral point was reached, a distinct opalescence appeared which was not affected by hydrochloric acid, but which was dissolved after a time by excess of caustic alkali.

The action of thiosulphuric acid upon arsenic acid appears, therefore, at least under the particular conditions studied, to be closely parallel to the action of thiosulphuric acid upon bichromic acid as described by Stiasny and Das.

For obvious reasons it is not likely that the reaction here noted, apparently for the first time, will afford the basis for a desirable volumetric method for use in the laboratory. It may be of value as a convenient means for reducing arsenic acid to arsenious acid preliminary to precipitation by hydrogen sulphide. As a basis for a field test, in default of anything better, it does offer some promise, and experiments in that direction are now under way.



# INDEX

	Page
<i>Abronia cycloptera</i> , in Tooele Valley, Utah.....	387
<i>salsa</i> , in Tooele Valley, Utah.....	387
Acid, arsenic, reduction to arsenious acid.....	515-517
estimation of.....	515
arsenious, reduction from arsenic acid.....	515-517
benzoic, in soil.....	357-358
metaoxytoluic, in soil.....	358-359
Adaptation in seedlings of Hopi maize.....	293-302
Adsorption by soils, selective.....	179-188
African cherry orange. See Citropsis.	
<i>Agromyza amoena</i> , syn. <i>A. pusilla</i> .	
<i>angulata</i> , resemblance to <i>A. pusilla</i> .	
<i>blanda</i> , syn. <i>A. pusilla</i> .	
<i>carbonaria</i> , relation to <i>A. pruinosa</i> .....	471
<i>coquilletti</i> , resemblance to <i>A. pusilla</i> .....	84
<i>diminuta</i> , syn. <i>A. pusilla</i> .	
<i>exilis</i> , syn. <i>A. pusilla</i> .	
<i>fuella</i> , syn. <i>A. pusilla</i> .	
<i>fusio</i> , syn. <i>A. pusilla</i> .	
<i>malampyga</i> , var. <i>marginalis</i> , resemblance to <i>A. pusilla</i> .....	85
<i>orborea</i> , syn. <i>A. pusilla</i> .	
<i>pruinosa</i> , cause of pith-ray flecks in <i>Betula nigra</i> .....	471-473
<i>pumila</i> , syn. <i>A. pusilla</i> .	
<i>pusilla</i> .....	67-74
distribution of.....	62
enemies of.....	76-83
food plants of.....	63-64
parasites of.....	76-83
<i>strigata</i> , syn. <i>A. pusilla</i> .	
<i>virens</i> , resemblance to <i>A. pusilla</i> .....	85
<i>Agropyron spicatum</i> , in Tooele Valley, Utah.....	378, 387
Alfalfa. See <i>medicago sativa</i> .	
Alkaloidal content of <i>Atropa belladonna</i> , variation in.....	129-146
Alkaloids, percentage in leaves of <i>Atropa belladonna</i> .....	132, 134-146
variation in leaves of <i>Atropa belladonna</i> .....	141-145
<i>Allenrolfea occidentalis</i> , in Tooele Valley, Utah.....	413
<i>Allium acuminatum</i> , in Tooele Valley, Utah.....	394
Almond, California desert. See <i>Prunus fasciculata</i> .	
desert. See <i>Prunus fasciculata</i> .	
Havard's. See <i>Prunus havardii</i> .	
Mexican. See <i>Prunus microphylla</i> .	
Nevada wild. See <i>Prunus andersonii</i> .	
Texas. See <i>Prunus minutiflora</i> .	
<i>Alternaria</i> sp., isolation from <i>Triticum sativum</i> .....	476
Alwood, W. B. (paper), Crystallization of Cream of Tartar in the Fruit of Grapes.....	513-514
America, South, potato weevils from.....	347-352
<i>Amsinckia tessellata</i> , in Tooele Valley, Utah.....	378



<i>Amygdalus andersonii</i> , syn. <i>Prunus andersonii</i> .	
<i>fasciculata</i> , syn. <i>Prunus fasciculata</i> .	
<i>fremonti</i> , syn. <i>Prunus eriogyna</i> .	
<i>glandulosa</i> , syn. <i>Prunus texana</i> .	
<i>microphylla</i> , syn. <i>Prunus microphylla</i> .	
<i>minutiflora</i> , syn. <i>Prunus minutiflora</i> .	
<i>texana</i> , syn. <i>Prunus texana</i> .	Page
<i>Anogra albicaulis</i> , in Tooele Valley, Utah . . . . .	378
<i>pallida</i> , in Tooele Valley, Utah . . . . .	378
<i>Antennaria dimorpha</i> , in Tooele Valley, Utah . . . . .	378
<i>Anthonomous grandis</i> , comparison with <i>A. grandis</i> , var. <i>thurberiae</i> . . . . .	91
<i>thurberiae</i> , danger from . . . . .	96
n. var. . . . .	90
Antigen from surra, used in diagnosis of dourine . . . . .	101-105
Apricot, desert. See <i>Prunus eriogyna</i> .	
<i>Arabis laevigata</i> , food plant of <i>Agromyza pusilla</i> . . . . .	64
<i>longirostris</i> , in Tooele Valley, Utah . . . . .	378
Arizona, occurrence of a cotton boll weevil in . . . . .	89-98
Arsenic acid. See Acid, arsenic.	
Arsenious acid. See Acid, arsenious.	
<i>Artemisia spinescens</i> , in Tooele Valley, Utah . . . . .	394
<i>tridentata</i> , in Tooele Valley, Utah . . . . .	377-387
<i>Aster pauciflorus</i> , in Tooele Valley, Utah . . . . .	405
<i>Astragalus arietinus</i> , in Tooele Valley, Utah . . . . .	378
<i>beckwithii</i> , in Tooele Valley, Utah . . . . .	378
<i>utahensis</i> , in Tooele Valley, Utah . . . . .	378
<i>Atriplex canescens</i> , in Tooele Valley, Utah . . . . .	378, 387
<i>confertifolia</i> , in Tooele Valley, Utah . . . . .	394-401
<i>nuttallii</i> , in Tooele Valley, Utah . . . . .	401
<i>spatiosa</i> , in Tooele Valley, Utah . . . . .	406
<i>Atropa belladonna</i> , percentage of alkaloids in leaves . . . . .	132, 134-146
variation in alkaloidal content . . . . .	129-146
variation of alkaloids in leaves . . . . .	141-145
<i>Avena sativa</i> , imperfect fungi isolated from . . . . .	475-489
inoculation with imperfect fungi . . . . .	476-481
<i>Bacterium aptatum</i> . . . . .	189-206
comparison with <i>B. phaseoli</i> . . . . .	208
comparison with <i>B. xanthochlorum</i> . . . . .	209-210
comparison with <i>Pseudomonas tenuis</i> . . . . .	206-208
n. sp. . . . .	206
Bacterium Causing a Disease of Sugar-Beet and Nasturtium Leaves, A (paper). . . . .	189-210
<i>Bacterium phaseoli</i> , comparison with <i>B. aptatum</i> . . . . .	208
<i>tumefaciens</i> , causal organism of crown-gall of <i>Carya illinoensis</i> . . . . .	334-337
<i>xanthochlorum</i> , comparison with <i>B. aptatum</i> . . . . .	209-210
Ballard, W. S., and Volck, W. H. (paper), Winter Spraying with Solutions of Nitrate of Soda. . . . .	437-444
<i>Balsamorhiza hirsuta</i> , in Tooele Valley, Utah . . . . .	378
<i>sagittata</i> , in Tooele Valley, Utah . . . . .	378
Barley. See <i>Hordeum vulgare</i> .	
Bean, hog. See <i>Hyoscyamus niger</i> .	
Beet, sugar. See <i>Beta vulgaris</i> .	
Belladonna. See <i>Atropa belladonna</i> .	
Bellflower. See <i>Campanula trachelium</i> .	

	Page
<i>Bellis perennis</i> , food plant of <i>Agromyza pusilla</i> .....	63
Benzene derivatives in soils.....	357-364
Benzoic acid. See Acid, benzoic.	
<i>Beta vulgaris</i> , bacterial disease of leaves.....	189-210
food plant of <i>Agromyza pusilla</i> .....	63
<i>Betula nigra</i> , cambium miner in.....	471-474
infestation by <i>Agromyza pruinosa</i> .....	473
Birch, river. See <i>Betula nigra</i> .	
Bladder senna. See <i>Colutea arborescens</i> .	
Blight, nursery, of <i>Carya illinoensis</i> .....	305-312
twig, of <i>Quercus prinus</i> .....	339-346
Boll weevil, cotton, occurrence in Arizona.....	89-98
<i>Brassica napus</i> , food plant of <i>Agromyza pusilla</i> .....	64
injury by <i>Agromyza pusilla</i> .....	74-75
oleracea, food plant of <i>Agromyza pusilla</i> .....	63
rapa, food plant of <i>Agromyza pusilla</i> .....	63
Briggs, L. J. et al., (paper), Indicator Significance of Vegetation, etc.....	365-418
<i>Bromus marginatus seminudus</i> , in Tooele Valley, Utah.....	394
tectorum, in Tooele Valley, Utah.....	378, 401
Brown, N. A. and Jamieson, C. O. (paper), Bacterium Causing a Disease of Sugar-Beet and Nasturtium Leaves.....	189-210
Buck, John M., Mohler, John R., and Eichhorn, Adolph (paper), Diagnosis of Dourine, etc.....	99-108
Cabbage. See <i>Brassica oleracea</i> .	
California desert almond. See <i>Prunus fasciculata</i> .	
soil, composition of <i>Triticum sativum</i> on.....	278-282
<i>Calliephialtes carbonarius</i> , relation to <i>Calliephialtes</i> sp.....	212
comstockii.....	214
messor.....	213-214
<i>Calliephialtes</i> Parasite of the Codling Moth, The (paper).....	211-238
<i>Calliephialtes pusio</i> .....	214
sp.....	211-235
Cambium Miner in River Birch, The (paper).....	471-474
<i>Campanula trachelium</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>Capsicum</i> sp., food plant of <i>Agromyza pusilla</i> .....	63
<i>Carduus scariosus</i> , in Tooele Valley, Utah.....	406
<i>Carya illinoensis</i> , diseases of.....	303-338
anthracnose of.....	319-330
crown-gall of.....	334-337
nursery, blight of.....	305-312
<i>Castanea dentata</i> , heart-rot of.....	117-119, 121, 127
inoculation with <i>Diplodia longispora</i> .....	341
twig blight of.....	339
pumila, heart-rot of.....	116-117, 119
<i>Castilleja linariaefolia</i> , in Tooele Valley, Utah.....	378, 387
Cells, tyloselike, occurrence in conifers.....	461-462
<i>Cerasus minutiflora</i> , syn. <i>Prunus minutiflora</i> .	
<i>Cercospora fusca</i> , causal organism of brown leaf-spot of <i>Carya illinoensis</i> .....	312-319
emend. sp.....	318-319
<i>Chaenactis douglasii</i> , in Tooele Valley, Utah.....	378
Chapin, R. M. (paper), Reduction of Arsenic Acid to Arsenious Acid by Thio- sulphuric Acid.....	515-517

	Page
Chemical characteristics of <i>Triticum sativum</i> , environmental influences on..	275-292
Cherry orange. See Citropsis.	
Chestnut. See <i>Castanea dentata</i> .	
Chinquapin. See <i>Castanea pumila</i> .	
<i>Chrysocharis ainsliei</i> , parasite of <i>Agromyza pusilla</i> .....	79
<i>farbesi</i> , parasite of <i>Agromyza pusilla</i> .....	79
<i>Chrysopsis villosa</i> , in Tooele Valley, Utah.....	378
<i>Chrysothamnus graveolens glabrata</i> , in Tooele Valley, Utah.....	405
<i>marianus</i> , in Tooele Valley, Utah.....	378, 394
<i>nauseosus albicaulis</i> , in Toole Valley, Utah.....	378, 387
<i>pumilus</i> , in Tooele Valley, Utah.....	378, 387
<i>Cirrospilus flavoviridis</i> , parasite of <i>Agromyza pusilla</i> .....	81
sp., parasite of <i>Agromyza pusilla</i> .....	82
Citropsis, a New Tropical African Genus Allied to Citrus (paper).....	419-436
<i>Citropsis articulata</i> , n. comb.....	433
<i>gabunensis</i> , n. comb.....	430-432
Citropsis, grafting of.....	435
hybridization of.....	435-436
<i>Citropsis mirabilis</i> , n. comb.....	432-433
Citropsis, new genus.....	421
<i>Citropsis Preussii</i> , n. comb.....	423-425
<i>Schweinfurthii</i> , n. comb.....	426-429
Citropsis, possible uses of.....	434
<i>Citrullus vulgaris</i> , food plant of <i>Agromyza pusilla</i> .....	63
Citrus, alliance to Citropsis.....	419-436
<i>Citrus articulata</i> , syn. <i>Citropsis articulata</i> .	
<i>Cavaleirei</i> , relation to <i>C. ichangensis</i> .....	11
<i>celebica</i> , relation to <i>C. ichangensis</i> .....	10
<i>histrix</i> , relation to <i>C. ichangensis</i> .....	10
Citrus Ichangensis, a Promising, Hardy, New Species from Southwestern China and Assam (paper).....	1-14
<i>Citrus ichangensis latipes</i> , n. subsp.....	11
<i>macroptera</i> , relation to <i>C. ichangensis</i> .....	10
<i>papuana</i> , relation to <i>C. ichangensis</i> .....	10
<i>Cladosporium gramineum</i> , isolated from <i>Avena sativa</i> .....	476
<i>Cleome serrulata</i> , in Tooele Valley, Utah.....	406
<i>Closteroceras utahensis</i> , parasite of <i>Agromyza pusilla</i> .....	81
Clover, red. See <i>Trifolium pratense</i> .	
sweet. See <i>Melilotus officinalis</i> .	
white. See <i>Trifolium repens</i> .	
zigzag. See <i>Trifolium medium</i> .	
Codling moth, Calliephialtes parasite of.....	211-238
Collins, G. N. (paper), Drought-Resisting Adaptation in Seedlings of Hopi Maize.....	293-302
<i>Colutea arborescens</i> , food plant of <i>Agromyza pusilla</i> .....	63
Complement fixation, diagnosis of dourine by.....	99-108
Complement-fixation test for dourine.....	105-107
<i>Coniothyrium caryogenum</i> , causal organism of kernel-spot in <i>Carya illinoensis</i> ..	330-334
n. sp.....	334
Cotton. See <i>Gossypium barbadense</i> .	
Cotton-boll weevil, occurrence in Arizona.....	89-98
<i>Cowania stansburiana</i> , in Tooele Valley, Utah.....	378
Cowpea. See <i>Vigna unguiculata</i> .	

	Page
<i>Crepis glauca</i> , in Tooele Valley, Utah.....	405
<i>occidentalis</i> , in Tooele Valley, Utah.....	378
Cream of tartar, crystallization in the fruit of grapes.....	513-514
Creosote, wood penetration of, affected by tyloses.....	464-467
Crown-gall of <i>Carya illinoensis</i> .....	334-337
<i>Cryptanthus multicaulis</i> , in Tooele Valley, Utah.....	394
Crystallization of Cream of Tartar in the Fruit of Grapes (paper).....	513-514
<i>Cryptanthus</i> sp., in Tooele Valley, Utah.....	378, 387
Cushman, R. A. (paper), Calliephialtes Parasite, etc.....	211-238
Cypress spurge. See <i>Euphorbia cyparissias</i> .	
<i>Cysticercus cellulosa</i> , comparison with <i>C. ovis</i> .....	31
confusion with <i>C. ovis</i> .....	15
syn. <i>Taenia ovis</i> .	
<i>ovipariens</i> , syn. <i>Taenia ovis</i> .	
<i>oviparus</i> , syn. <i>Taenia ovis</i> .	
<i>ovis</i> . See also <i>Taenia ovis</i> .	
<i>Cysticercus Ovis</i> , the Cause of Tapeworm Cysts in Mutton (paper).....	15-58
<i>Cysticercus tenuicollis</i> , comparison with <i>C. ovis</i> .....	32-33
confusion with <i>C. ovis</i> .....	17
syn. <i>Taenia ovis</i> .	
Cysts, tapeworm, in mutton, <i>Cysticercus ovis</i> , cause of.....	15-58
Dahlberg, A. O., and Rogers, L. A. (paper), Origin of Some of the Streptococci, etc.....	491-511
Daisy, garden. See <i>Bellis perennis</i> .	
Dandelion. See <i>Taraxacum geniculata</i> .	
<i>Delphinium burkei</i> , in Tooele Valley, Utah.....	378
<i>Derostenus arizonensis</i> , parasite on <i>Agromyza pusilla</i> .....	80
<i>diastatae</i> , parasite on <i>Agromyza pusilla</i> .....	80
<i>functiventus</i> , parasite on <i>Agromyza pusilla</i> .....	80
<i>pictipes</i> , parasite on <i>Agromyza pusilla</i> .....	80
<i>varipes</i> , parasite on <i>Agromyza pusilla</i> .....	81
Desert apricot. See <i>Prunus eriogyna</i> .	
Diagnosis of Dourine by Complement Fixation, The (paper).....	99-108
<i>Diaulinopsis callichroma</i> , parasite of <i>Agromyza pusilla</i> .....	81
sp., parasite of <i>Agromyza pusilla</i> .....	82
<i>Diaulinus begini</i> , parasite of <i>Agromyza pusilla</i> .....	78
<i>websteri</i> , parasite of <i>Agromyza pusilla</i> .....	79
<i>Dibothriocephalus</i> spp., comparison with <i>Taenia ovis</i> .....	35
<i>Diplodia longispora</i> , causal organism of twig blight of <i>Quercus prinus</i> .....	345-346
<i>Dipylidium caninum</i> , comparison with <i>Taenia ovis</i> .....	34
Disease, bacterial, of leaves of <i>Beta vulgaris</i> and <i>Tropaeolum majus</i> .....	189-210
Diseases of <i>Carya illinoensis</i> .....	303-338
<i>Distichlis spicata</i> , in Tooele Valley, Utah.....	405
<i>Dodecatheon</i> sp., in Tooele Valley, Utah.....	405
Dourine, complement-fixation test for.....	105-107
diagnosis by complement fixation.....	99-108
<i>Draba</i> sp., in Tooele Valley, Utah.....	378
Drought-Resisting Adaptation in Seedlings in Hopi Maize, A (paper).....	293-302
<i>Echinococcus granulosus</i> , comparison with <i>Taenia ovis</i> .....	34
Eichhorn, Adolph, Mohler, John R., and Buck, John M. (paper), Diagnosis of Dourine, etc.....	99-108
Elder, European. See <i>Sambucus nigra</i> .	

	Page
<i>Elymus condensatus</i> , in Tooele Valley, Utah.....	401
<i>Emplectocladus andersonii</i> , syn. <i>Prunus andersonii</i> . <i>fasciculatus</i> , syn. <i>Prunus fasciculata</i> .	
<i>Emplectocladus</i> , subgenus of <i>Prunus</i> .....	153
Enemies of <i>Agromyza pusilla</i> .....	76-83
<i>See also</i> Parasite.	
<i>Entedoninae</i> , parasite of <i>Agromyza pusilla</i> .....	82
Environmental Influences on the Physical and Chemical Characteristics of Wheat (paper).....	275-292
<i>Ephialtes carbonarius</i> , syn. <i>Calliephialtes carbonarius</i> . <i>comstockii</i> , syn. <i>Calliephialtes comstockii</i> . <i>messor</i> . <i>See Calliephialtes messor</i> . <i>pusio</i> , syn. <i>Calliephialtes pusio</i> .	
<i>Erigeron pumilus</i> , in Tooele Valley, Utah.....	378
<i>Eriocoma cuspidata</i> , in Tooele Valley, Utah.....	378, 387
<i>Eriogonum cernuum</i> , in Tooele Valley, Utah.....	387
<i>kearneyi</i> , in Tooele Valley, Utah.....	387
<i>ovalifolium</i> , in Tooele Valley, Utah.....	378, 387
<i>Erodium cicutarium</i> , in Tooele Valley, Utah.....	378, 387, 389
Errata.....	IV
<i>Erysimum asperim</i> , in Tooele Valley, Utah.....	401
<i>Erythraea arizonica</i> , in Tooele Valley, Utah.....	406
<i>Erythraeus</i> , enemy of <i>Agromyza pusilla</i> .....	83
<i>Eucoila bunteri</i> , parasite of <i>Agromyza pusilla</i> .....	82
<i>Euphorbia cyparissias</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>Euprunus</i> , subgenus of <i>Prunus</i> .....	153
<i>Eurotia lanata</i> , in Tooele Valley, Utah.....	387, 394
Feces, streptococci from.....	492
Fenugreek. <i>See Trigonella foenum-graecum</i> .	
Fermentation, caused by streptococci.....	504-505
<i>Festuca octoflora hirtella</i> , in Tooele Valley, Utah.....	378
Fixation, complement, diagnosis of dourine by.....	99-108
Flecks, pith-ray, in <i>Betula nigra</i> .....	471-473
<i>Fomes lobatus</i> , cause of heart-rot of <i>Quercus</i> .....	110
Foot-Rot of the Sweet Potato, The (paper).....	251-274
Foreword.....	i
Fungi, imperfect, isolation from <i>Triticum sativum</i> , <i>Avena sativa</i> , and <i>Hordeum</i> <i>vulgare</i> .....	475-489
<i>Fusarium culmorum</i> , isolation from <i>Avena sativa</i> .....	476
<i>invale</i> , relation to infection of cereals.....	486
<i>roseum</i> , relation to <i>F. culmorum</i> and to <i>Gibberella saubineti</i> .....	485
<i>rubiginosum</i> , syn. of <i>F. culmorum</i> .	
<i>Galeopsis tetrahit</i> , food plant of <i>Agromyza pusilla</i> .....	63
Galloway, B. T. (paper), Foreword.....	i
<i>Gaura parviflora</i> , in Tooele Valley, Utah.....	378
Gerry, E. (paper), Tyloses; Their Occurrence, etc.....	445-469
<i>Gibberella saubineti</i> , relation to <i>Fusarium roseum</i> .....	485
<i>Gilia leptomeria</i> , in Tooele Valley, Utah.....	387
<i>pungens</i> , in Tooele Valley, Utah.....	387
<i>Glaux maritima</i> , in Tooele Valley, Utah.....	405
<i>Glomerella cingulata</i> , causal organism of anthracnose of <i>Carya illinoensis</i> .....	319-330

	Page
<i>Gossypium barbadense</i> , food plant of <i>Agromyza pusilla</i> .....	64
injury by <i>Agromyza pusilla</i> .....	75-76
Grapes, crystallization of cream of tartar in the fruit of.....	513-514
Grass, salt. See <i>Distichlis spicata</i> .	
<i>Grayia spinosa</i> , in Tooele Valley, Utah.....	387
Greasewood-shadscale. See <i>Sarcobatus</i> spp. and <i>Atriplex</i> spp.	
Greene, C. T. (paper), Cambium Miner in River Birch.....	471-474
<i>Gutierrezia sarothrae</i> in Tooele Valley, Utah.....	378, 389, 401
<i>Gymnosporangium chinensis</i> , n. sp., on <i>Juniperus chinensis</i> .....	354
<i>haraenum</i> , relation to <i>G. chinensis</i> .....	354
<i>japonicum</i> , relation to <i>G. chinensis</i> .....	354
<i>Gymnosporangium</i> , species from Japan.....	353-356
<i>Halerpestes cymbalaria</i> in Tooele Valley, Utah.....	405
Hardwood, heart-rot, especially of <i>Quercus</i> .....	109-128
tyloses in.....	451
Harter, L. L. (paper), Foot-Rot of the Sweet Potato.....	251-274
Harvard's almond. See <i>Prunus harvardii</i> .	
Heart-rot, of hardwood trees, especially of <i>Quercus</i> .....	109-128
<i>Helminthosporium avenae</i> , relation to <i>H. gramineum</i> .....	484
<i>gramineum</i> , isolation from <i>Triticum sativum</i> and <i>Hordeum vulgare</i> .....	475-476
stunting of roots of <i>Triticum sativum</i> by.....	481
<i>teres</i> , relation to <i>H. gramineum</i> .....	484
Henbane. See <i>Hyoscyamus niger</i> .	
<i>Hesperethusa crenulata</i> , syn. <i>Limonia acidissima</i> .	
Hickory, pignut. See <i>Hicoria glabra</i> .	
<i>Hicoria glabra</i> , tyloses in.....	451
Hopi maize. See maize, Hopi.	
<i>Hordeum jubatum</i> , in Tooele Valley, Utah.....	406
<i>vulgare</i> , imperfect fungi isolated from.....	475-489
inoculation with imperfect fungi.....	476-481
Hybrids, <i>Prunus texana</i> .....	161-164
<i>Hydnum erinaceus</i> , cause of hollow-producing rot in <i>Quercus</i> .....	109-112, 121
<i>Hyoscyamus niger</i> , food plant of <i>Agromyza pusilla</i> .....	63
Imperfect fungi. See Fungi, imperfect.	
Indicator Significance of Vegetation in Tooele Valley, Utah (paper).....	365-418
Individual Variation in the Alkaloidal Content of Belladonna Plants (paper)..	129-146
Influences, environmental, on the physical and chemical characteristics of	
<i>Triticum sativum</i> .....	275-292
Ingram, D. E. (paper), Twig Blight of <i>Quercus</i> Prinus, etc.....	339-346
<i>Ipomoea batatas</i> , foot-rot of.....	251-274
<i>Iris</i> sp., in Tooele Valley, Utah.....	405
<i>Iva auxillaris</i> , in Tooele Valley, Utah.....	405
Jamieson, C. O., and Brown, N. A. (paper), Bacterium Causing a Disease of	
Sugar-Beet and Nasturtium Leaves.....	189-210
Japan, species of <i>Gymnosporangium</i> from.....	353-356
Johnson, E. C. (paper), Study of Some Imperfect Fungi Isolated from Wheat,	
Oat, and Barley Plants.....	475-489
<i>Juncus balticus</i> , in Tooele Valley, Utah.....	405
<i>Juniperus utahensis</i> , in Tooele Valley, Utah.....	387

	Page
Kansas soil, composition of <i>Triticum sativum</i> on.....	278-282
Kearney, T. H., Briggs, L. J., Shantz, H. L., McLane, J. W., and Piemeisel, R. L. (paper), Indicator Significance of Vegetation, etc.....	365-418
Kellerman, M., and Swingle, W. T. (paper), Citropsis, etc.....	419-436
Kernel-spot of <i>Carya illinoensis</i> .....	330-335
<i>Kochia vestita</i> , in Tooele Valley, Utah.....	388-394, 401
<i>Lappula caerulea</i> , in Tooele Valley, Utah.....	378
<i>cupulata</i> , in Tooele Valley, Utah.....	378
<i>occidentalis</i> , in Tooele Valley, Utah.....	378, 387, 394, 401
sp., in Tooele Valley, Utah.....	387
<i>Lappula subdecumbens</i> , in Tooele Valley, Utah.....	378
<i>Lathyrus odoratus</i> , food plant of <i>Agromyza pusilla</i> .....	64
<i>Layia glandulosa</i> , in Tooele Valley, Utah.....	378, 387
Leaf-miner, serpentine.....	59-88
Leaf-spot, brown, of <i>Carya illinoensis</i> .....	312-319
LeClerc, J. A., and Yoder, P. A., (paper) Environmental Influences on the Physical and Chemical Characteristics of Wheat.....	275-292
<i>Lepidium jonesii</i> , in Tooele Valley, Utah.....	394
<i>pubecarpum</i> , in Tooele Valley, Utah.....	387
sp., in Tooele Valley, Utah.....	389
<i>Leucelene ericoides</i> , in Tooele Valley, Utah.....	378
<i>Limonia acidissima</i> , relation to Citropsis.....	420
<i>Demeusei</i> .....	434
<i>gabunensis</i> , syn. <i>Citropsis gabunensis</i> .	
<i>Lacourtiana</i> , syn. <i>Citropsis gabunensis</i> .	
<i>mirabilis</i> , syn. <i>Citropsis mirabilis</i> .	
<i>Poggei</i> , syn. <i>Citropsis Schweinfurthii</i> .	
var. <i>latialata</i> .....	434
<i>Preussii</i> , syn. <i>Citropsis Preussii</i> .	
<i>Schweinfurthii</i> , syn. <i>Citropsis Schweinfurthii</i> .	
<i>ugandensis</i> , syn. <i>Citropsis Schweinfurthii</i> .	
Long, W. H. (paper), Polyporus Dryadeus, etc.....	239-250
Undescribed Species of Gymnosporangium, etc.....	353-356
Three Undescribed Heart-Rots of Hardwood Trees, etc.....	109-128
McLane, J. W., et al. (paper), Indicator Significance of Vegetation, etc.....	365-418
<i>Machaeranthera canescens</i> , in Tooele Valley, Utah.....	401
Maize, germination of varieties when planted at different depths.....	296-298
Hopi, drought-resisting adaptation of.....	293-302
mesocotyl of.....	294-295
Mallow, common. See <i>Malva rotundifolia</i> .	
<i>Malva rotundifolia</i> , food plant of <i>Agromyza pusilla</i> .....	64
<i>Malvastrum coccineum</i> , in Tooele Valley, Utah.....	378
Maryland soil, composition of <i>Triticum sativum</i> on.....	278-282
Mason, S. C. (paper), Pubescent-Fruited Species of <i>Prunus</i> , etc.....	147-178
Meadow queen. See <i>Spiraea almaria</i> .	
Measles, sheep.....	15
eradication of.....	51-52
geographic distribution of.....	48
<i>Medicago sativa</i> , food plant of <i>Agromyza pusilla</i> .....	59-60
<i>Melilotus alba</i> , in Tooele Valley, Utah.....	406
<i>officinalis</i> , food plant of <i>Agromyza pusilla</i> .....	64
<i>Mentzelia dispersa</i> , in Tooele Valley, Utah.....	378
<i>laevicaulis</i> , in Tooele Valley, Utah.....	378

	Page
<i>Mesocestoides</i> spp., comparison with <i>Taenia ovis</i> .....	35
Metaoxytoluic acid. See Acid, metaoxytoluic.	
Mexican almond. See <i>Prunus microphylla</i> .	
Milk, origin of some of the streptococci.....	491-511
streptococci from.....	492
Miner, cambium, in <i>Betula nigra</i> .....	471-474
leaf, serpentine.....	59-88
Mohler, John R., Eichhorn, Adolph, and Buck, John M. (paper), Diagnosis of Dourine, etc.....	99-108
Moth, codling, <i>Calliephialtes</i> parasite of.....	211-238
Mouth, streptococci from.....	492
<i>Multiceps multiceps</i> , comparison with <i>Taenia ovis</i> .....	34-35
<i>serialis</i> , comparison with <i>Taenia ovis</i> .....	34
Mutton, <i>Cysticercus ovis</i> , cause of tapeworm cysts in.....	15-58
Mustard, hedge. See <i>Sisymbrium officinale</i> .	
Nasturtium. See <i>Tropaeolum majus</i> .	
Nettle, hedge. See <i>Stachys sylvanica</i> .	
hemp. See <i>Galeopsis tetrahit</i> .	
Nevada wild almond. See <i>Prunus andersonii</i> .	
New Potato Weevils from Andean South America (paper).....	347-352
<i>Nicotiana</i> sp., food plant of <i>Agromyza pusilla</i> .....	64
Nitrate of soda, winter spraying with.....	437-444
Norway pine. See <i>Pinus resinosa</i> .	
Nursery-blight of <i>Carya illinoensis</i> .....	305-312
Oak, blackjack. See <i>Quercus marilandica</i> .	
scarlet. See <i>Quercus coccinea</i> .	
Texan. See <i>Quercus texana</i> .	
valley. See <i>Quercus lobata</i> .	
white. See <i>Quercus alba</i> .	
See also <i>Quercus</i> spp.	
Oats. See <i>Avena sativa</i> .	
Occurrence of a Cotton Boll Weevil in Arizona, The (paper).....	89-98
<i>Ononis repens</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>spinosa</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>Ophidiotaenia punica</i> , comparison with <i>Taenia ovis</i> .....	35
<i>Opius agromyzae</i> , parasite of <i>Agromyza pusilla</i> .....	82
<i>aridus</i> , parasite of <i>Agromyza pusilla</i> .....	82
<i>brunneipes</i> , parasite of <i>Agromyza pusilla</i> .....	82
<i>suturalis</i> , parasite of <i>Agromyza pusilla</i> .....	82
<i>Opuntia</i> sp., in Tooele Valley, Utah.....	378, 389, 394
Orange, African cherry. See <i>Citropsis</i> .	
<i>Oreocarya shantzii</i> , in Tooele Valley, Utah.....	394
<i>Orthocarpus tolmiei</i> , in Tooele Valley, Utah.....	406
Origin of Some of the Streptococci Found in Milk, The (paper).....	491-511
<i>Oscinus brassicae</i> , syn. <i>Agromyza pusilla</i> .	
<i>trifolii</i> , syn. <i>Agromyza pusilla</i> .	
<i>Pachylophus marginatus</i> , in Tooele Valley, Utah.....	378
Parasite, <i>Calliephialtes</i> , of the codling moth.....	211-238
of <i>Agromyza pusilla</i> .....	76-83
root, on <i>Quercus</i> spp.....	239-250
sheep-measle. See <i>Taenia ovis</i> .	



	Page
Parker, E. G. (paper), Selective Adsorption by Soils .....	179-188
Parks, T. H., and Webster, F. M. (paper), Serpentine Leaf-Miner .....	59-88
Pea, sweet. <i>See Lathyrus odoratus</i> .	
Peach, wild. <i>See Prunus texana</i> .	
Pecan. <i>See Carya illinoensis</i> .	
Penarmeniaca, n. sect. ....	154
Pepper. <i>See Capsicum</i> sp.	
<i>Phacelia linearis</i> , in Tooele Valley, Utah .....	378
<i>Phlox longifolia</i> , in Tooele Valley, Utah .....	378
<i>Phyllosticta caryogena</i> , syn. of <i>P. caryae</i> .	
<i>caryae</i> , causal organism of nursery-blight of <i>Carya illinoensis</i> .....	305-312
<i>convexula</i> , growth with <i>Glomerella cingulata</i> .....	329
Physical characteristics of <i>Triticum sativum</i> , environmental influences on. ....	275-292
Piemeisel, R. L., et al. (paper), Indicator Significance of Vegetation, etc. ....	365-418
Pierce, W. D. (paper), New Potato Weevils, etc. ....	347-352
Occurrence of a Cotton Boll Weevil in Arizona. ....	89-98
Piloprunus, n. sect. ....	153-154
Pine, Norway. <i>See Pinus resinosa</i> .	
Piñon. <i>See Pinus edulis</i> .	
Piñon pine. <i>See Pinus edulis</i> .	
<i>Pinus edulis</i> , tyloses lacking in. ....	460
<i>resinosa</i> , tyloses in. ....	460
Pith-ray flecks in <i>Betula nigra</i> .....	471-473
<i>Plantago</i> sp., food plant of <i>Agromyza pusilla</i> .....	64
Plantain. <i>See Plantago</i> sp.	
<i>Plenodomus destruens</i> , causal organism of foot-rot of <i>Ipomoea batatas</i> .....	253-273
<i>Pleutotropis rugosithorax</i> , parasite of <i>Agromyza pusilla</i> .....	82
<i>Poa nevadensis</i> , in Tooele Valley, Utah .....	405
<i>sandbergii</i> , in Tooele Valley, Utah .....	378, 389, 394
sp. ....	401
<i>Polyporus berkeleyi</i> , causal organism of string and ray rot, in <i>Quercus</i> . .	110-112, 122-125
in <i>Quercus alba</i> .....	122-125
in <i>Quercus velutina</i> . ....	123
<i>corruscans</i> , syn. <i>P. dryophilus</i> .	
<i>Polyporus Dryadeus</i> , a Root Parasite on the Oak (paper) .....	239-250
<i>Polyporus dryophilus</i> , causal organism of heart-rot in <i>Quercus</i> .....	109-112
confusion with <i>P. dryadeus</i> . ....	239-241
<i>freisii</i> , syn. <i>P. dryophilus</i> .	
<i>frondosus</i> , causal organism of straw-colored rot in <i>Quercus</i> . ....	110-112, 125-127
occurrence on <i>Quercus digitata</i> .....	127
<i>fulvus</i> , syn. <i>P. dryophilus</i> .	
<i>pilotae</i> , causal organism of pocketed or piped rot. ....	110-112, 114-122
in <i>Quercus alba</i> .....	110-112, 114-115
in <i>Quercus coccinea</i> .....	115-116
in <i>Castanea dentata</i> .....	117-118
in <i>Castanea pumila</i> .....	116-117
in <i>Quercus texana</i> .....	116
<i>rheades</i> , syn. <i>P. dryophilus</i> .	
<i>sulphureus</i> , causal organism of brown, checked rot of <i>Quercus</i> .....	109-112
<i>vulpinus</i> , syn. <i>P. dryophilus</i> .	
Potassium bitartrate, crystals in fruit .....	513-514
Potato. <i>See Solanum tuberosum</i> .	
Potato, sweet. <i>See Ipomoea batatas</i> .	

	Page
Premnotrypes, new genus. ....	348
<i>Premnotrypes solani</i> , n. sp. ....	348-349
Presence of Some Benzene Derivatives in Soils, The (paper). ....	357-364
<i>Prunus</i> , classification of. ....	153-154
<i>Prunus andersonii</i> . ....	164-166
<i>eriogyna</i> , n. sp. ....	166-170
<i>fasciculata</i> . ....	170-172
<i>fremonti</i> , syn. <i>Prunus eriogyna</i> .	
<i>glandulosa</i> , syn. <i>P. texana</i> .	
<i>havardii</i> , n. comb. ....	176-177
<i>Hookeri</i> , syn. <i>P. texana</i> .	
<i>microphylla</i> . ....	174-176
<i>minutiflora</i> . ....	172-174
<i>Prunus</i> , pubescent-fruited species of the Southwestern States. ....	147-178
<i>Prunus texana</i> ....	154-164
hybrids. ....	161-164
<i>Pseudomanas tenuis</i> , comparison with <i>Bacterium apatum</i> . ....	206-208
<i>Psoralea lanceolata</i> , in Tooele Valley, Utah. ....	387
<i>Pteromalus</i> sp., parasite of <i>Agromyza pusilla</i> . ....	82
<i>Ptilocalais nutans</i> , in Tooele Valley, Utah. ....	378
Pubescent-Fruited Species of <i>Prunus</i> of the Southwestern States, The (paper). ....	147-178
<i>Puccinellia airoides</i> , in Tooele Valley, Utah. ....	405
<i>Purshia tridentata</i> , in Tooele Valley, Utah. ....	387
<i>Quercus alba</i> , heart-rot of. ....	109-112, 114-115, 122-127
inoculation with <i>Diplodia longispora</i> . ....	341
root-rot of. ....	245-246
twig blight of. ....	339
<i>coccinea</i> , heart-rot of. ....	114-116
host for <i>Diplodia longispora</i> . ....	345
<i>gambelli</i> , inoculations with <i>Diplodia longispora</i> . ....	341
<i>lobata</i> , inoculation with <i>Diplodia longispora</i> . ....	341
tyloses in. ....	451
<i>marilandica</i> , tyloses in. ....	451
<i>minor</i> , inoculation with <i>Diplodia longispora</i> . ....	341
root-rot of. ....	245
<i>nigra</i> , root-rot of. ....	245
<i>prinus</i> , root-rot of. ....	245
twig blight of. ....	339-346
<i>rubra</i> , inoculation with <i>Diplodia longispora</i> . ....	341
spp., heart-rots of. ....	109-128
root parasite on. ....	239-250
root-rot caused by <i>Polyporus dryadeus</i> . ....	245-247
<i>texana</i> , heart-rot of. ....	116, 119
inoculation with <i>Diplodia longispora</i> . ....	341
root-rot of. ....	245
<i>velutina</i> , root-rot of. ....	245
<i>virginiana</i> , inoculation with <i>Diplodia longispora</i> . ....	341
Radish. See <i>Raphanus sativus</i> .	
Rand, F. V. (paper), Some Diseases of Pecans. ....	303-338
Ransom, B. H. (paper), Cysticercus Ovis, the Cause of Tapeworm Cysts in Mutton. ....	15-58
Rape. See <i>Brassica napus</i> .	

	Page
<i>Raphanus sativus</i> , food plant of <i>Agromyza pusilla</i> .....	63
Reduction of Arsenic Acid to Arsenious Acid by Thiosulphuric Acid (paper). 515-517	
Rest-harrow. See <i>Ononis</i> spp.	
<i>Rhigopsidius tucumanus</i> .....	347, 350-351
River birch. See <i>Betula nigra</i> .	
Rock cress, smooth. See <i>Arabis laevigata</i> .	
Rogers, L. A., and Dahlberg, A. O. (paper), Origin of Some of the Streptococci Found in Milk.....	491-511
Root parasite on <i>Quercus</i> spp. ....	239-250
Root-rot of oak. See Root-rot of <i>Quercus</i> spp.	
of <i>Quercus</i> spp. ....	245-247
Rot, brown, checked.....	109-114
butt, types found in <i>Quercus alba</i> .....	111-112
checked. See Rot, brown, checked.	
heart. See Heart-rot.	
hollow-producing.....	109-112
piped. See Rot, pocketed or piped.	
pocketed or piped.....	109-112, 113-122
ray. See Rot, string and ray.	
root. See Root-rot.	
straw-colored.....	110-112, 125-127
string and ray.....	110-112, 122-125
Rye. See <i>Secale cereale</i> .	
Sagebrush. See <i>Artemisia tridentata</i> .	
<i>Salicornia rubra</i> , in Tooele Valley, Utah.....	406
Salt grass. See <i>Distichlis spicata</i> .	
<i>Sambucus nigra</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>Sarcobatus vermiculatus</i> , in Tooele Valley, Utah.....	387, 401
<i>Secale cereale</i> , inoculation with imperfect fungi.....	476-478
Selective Adsorption by Soils (paper).....	179-188
<i>Senecio uintahensis</i> , in Tooele Valley, Utah.....	378, 387
Senna, bladder. See <i>Colutea arborescens</i> .	
Serpentine Leaf-Miner, The (paper).....	59-88
Shadscale. See <i>Atriplex confertifolia</i> .	
Shantz, H. L., et al., (paper) Indicator Significance of Vegetation, etc.....	365-418
Sheep measles. See Measles, sheep.	
number affected with sheep measles.....	16
Shorey, E. C. (paper), Presence of Some Benzene Derivatives in Soils.....	357-364
Sievers, A. F. (paper), Individual Variation in the Alkaloidal Content of Belladonna Plants.....	129-146
<i>Sisymbrium officinale</i> , food plant of <i>Agromyza pusilla</i> .....	64
<i>Sitanion jubatum</i> , in Tooele Valley, Utah.....	378
minus, in Tooele Valley, Utah.....	394, 401
Soda, nitrate of, winter spraying with.....	437-444
Softwood, tyloses in.....	458-461
Soil, benzene derivatives in.....	357-364
benzoic acid in.....	357-358
infected with <i>Helminthosporium gramineum</i> , injury to <i>Triticum sativum</i> by.....	481
metaoxytoluic acid in.....	358-359
selective adsorption by.....	179-188
vanillin in.....	359-362
<i>Solanum tuberosum</i> , food plant of <i>Agromyza pusilla</i> .....	63

	Page
Some Diseases of Pecans (paper).....	303-338
<i>Sonchus oleraceus</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>Sophia filipes</i> , in Tooele Valley, Utah.....	378
<i>pinnata</i> , in Tooele Valley, Utah.....	378, 401
South America, potato weevils from.....	347-352
Southwestern States, pubescent-fruited species of <i>Prunus</i> from.....	147-178
<i>Spartina gracilis</i> , in Tooele Valley, Utah.....	405
<i>Sphaerella convexula</i> , relation to <i>Phyllosticta convexula</i> .....	329
<i>Sphaeria convexula</i> , syn. <i>Sphaerella convexula</i> .	
<i>Sphaerostigma pubens</i> , in Tooele Valley, Utah.....	389
Spinach. See <i>Spinacia oleracea</i> .	
<i>Spinacia oleracea</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>Spiraea ulmaria</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>Sporobolus airoides</i> , in Tooele Valley, Utah.....	405
Spraying, dormant, stimulation by.....	437-444
winter, with nitrate of soda.....	437-444
<i>Stachys sylvantrica</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>Stipa comata</i> , in Tooele Valley, Utah.....	378, 387
Streptococci, action on litmus milk.....	503
correlation of physiological characters of.....	497-502
fermentation of carbohydrates caused by.....	504-505
origin of some found in milk.....	491-511
relation of physiological groups to known species of.....	507-508
<i>Streptococcus pyogenes</i> , relation to physiological groups of cultures.....	507-508
Study of Some Imperfect Fungi Isolated from Wheat, Oat, and Barley Plants, A (paper).....	475-489
<i>Suaeda erecta</i> , in Tooele Valley, Utah.....	406
<i>intermedia</i> , in Tooele Valley, Utah.....	401
<i>moquini</i> , in Tooele Valley, Utah.....	401
Sugar beet. See <i>Beta vulgaris</i> .	
Surra, antigen from, used in diagnosis of dourine.....	101-105
Sweet potato. See <i>Ipomoea batatas</i> .	
Swingle, W. T., paper, <i>Citrus Ichangensis</i> , etc.....	1-14
and Kellerman, M., paper, <i>Citropsis</i> , etc.....	419-436
<i>Sympha agromyzae</i> , parasite of <i>Agromyza pruinosa</i> .....	474
<i>Sympiesis</i> sp., parasite of <i>Agromyza pusilla</i> .....	82
<i>Taenia balaniceps</i> , comparison with <i>T. ovis</i> .....	35
<i>brachymosa</i> , comparison with <i>T. ovis</i> .....	35-36
<i>brauni</i> , comparison with <i>T. ovis</i> .....	35
<i>coenurus</i> . See <i>Multiceps multiceps</i> .	
<i>echinococcus</i> . See <i>Echinococcus granulosus</i> .	
<i>hydatigena</i> , comparison with <i>T. ovis</i> .....	33-34
<i>krabbei</i> , comparison with <i>T. ovis</i> .....	36-37
<i>marginata</i> . See <i>T. hydatigena</i> .	
<i>ovis</i> , comparison with other species.....	31-39
life history of.....	20-28
<i>pisiformis</i> , comparison with <i>T. ovis</i> .....	34
<i>serialis</i> . See <i>Multiceps serialis</i> .	
<i>serrata</i> . See <i>T. pisiformis</i> .	
Tapeworm cysts in mutton, <i>Cysticercus ovis</i> , cause of.....	15-58
of dog. See <i>Taenia ovis</i> .	
<i>Taraxacum geniculata</i> , food plant of <i>Agromyza pusilla</i> .....	63
Tartar, cream of. See Cream of tartar.	

	Page
<i>Tetradymia glabrata</i> , in Tooele Valley, Utah.....	394
<i>inermis</i> , in Tooele Valley, Utah.....	378
<i>nuttallii</i> , in Tooele Valley, Utah.....	401
<i>spinosa</i> , in Tooele Valley, Utah.....	394
Texas almond. See <i>Prunus minutiflora</i> .	
<i>Thalesia fasciculata</i> , in Tooele Valley, Utah.....	378
<i>Thelypodium elegans</i> , in Tooele Valley, Utah.....	394
Thistle, sow. See <i>Sonchus oleraceus</i> .	
Three Undescribed Heart-Rots of Hardwood Trees, Especially of Oak (paper). 109-128	
<i>Thurberia thespesioides</i> , host for cotton-boll weevil.....	92
Tobacco. See <i>Nicotiana</i> sp.	
Tooele Valley, Utah, classification of types of vegetation in.....	374-375
climate of.....	369-370
correlation between types of vegetation and productivity of land in.....	412-415
determination of soil-moisture content in.....	367
moisture equivalent in.....	367
wilting coefficient in.....	367
salt content of.....	367-369
geology and topography of.....	370-371
grass-flat communities in.....	405-406
greasewood-shadscale association in.....	400-405
indicator significance of vegetation in.....	365-418
Kochia association in.....	388-394
sagebrush association in.....	377-386
saline conditions of.....	371-374
salt-flat communities in.....	408-412
sand-hill mixed association in.....	386-388
shadscale association in.....	394-400
<i>Townsendia watsonii</i> , in Tooele Valley, Utah.....	394
<i>Trifolium medium</i> , food plant of <i>Agromyza pusilla</i> .....	63
<i>pratense</i> , food plant of <i>Agromyza pusilla</i> .....	64
<i>repens</i> , food plant of <i>Agromyza pusilla</i> .....	63-64
<i>Triglochin maritima</i> , in Tooele Valley, Utah.....	405
<i>palustris</i> , in Tooele Valley, Utah.....	405
<i>Trigonella foenum-graecum</i> , food plant of <i>Agromyza pusilla</i> .....	64
<i>Triphelps</i> sp., enemy of <i>Agromyza pusilla</i> .....	83
<i>Triticum sativum</i> , ash of.....	287
chemical constituents of.....	284-288
composition on different plats of soil in Kansas, California, and Maryland.....	278
composition when grown on soil from California, Kansas, and Maryland in each of the three States.....	280
correlation between physical properties and chemical constituents of.....	288
environmental influences on the physical and chemical characteristics of.....	275-292
fat of.....	286
fiber of.....	286-287
flinty grains of.....	283-284
gliadin in protein of.....	286
imperfect fungi isolated from.....	475-489
inoculation with imperfect fungi.....	476-478
pentosans of.....	287
phosphoric-acid content of.....	287
physical and chemical characteristics of.....	275-292
potash of.....	288
protein of.....	284-286

	Page
<i>Triticum sativum</i> , stunting of roots by <i>Helminthosporium gramineum</i> .....	481
sugars of.....	287
weight of 1 bushel of.....	283
weight of 1,000 grains of.....	283
<i>Tropaeolum majus</i> , bacterial disease of leaves.....	189-210
food plant of <i>Agromyza pusilla</i> .....	63
<i>Trypanosoma equiperdum</i> , causal organism of dourine.....	99
Turnip. See <i>Brassica rapa</i> .	
Twig Blight of <i>Quercus Prinus</i> and Related Species, A (paper).....	339-346
Tyloselike cells, occurrence in conifers.....	461-462
Tyloses, development of.....	446
occurrence in conifers.....	459-461
in hardwoods.....	451
in softwoods.....	458-459
practical significance of.....	462-468
relation of parenchyma development to.....	448
to creosote penetration of wood.....	464-467
to durability of wood.....	462-464
to water-logging of wood.....	467-468
Tyloses: Their Occurrence and Practical Significance in Some American Woods (paper).....	445-469
<i>Trypopermnon latithorax</i> , n. sp.....	350
<i>Trypopermnon</i> , new genus.....	349
Udder, streptococci from.....	492
Undescribed heart-rots of hardwood trees.....	109-128
Species of <i>Gymnosporangium</i> from Japan, An (paper).....	353-356
Utah, indicator significance of vegetation in Tooele Valley.....	365-418
Vanillin in soil.....	359-362
Variation in the alkaloidal content of <i>Atropa belladonna</i> .....	129-146
Vegetation in Tooele Valley, Utah, indicator significance of.....	365-418
Vetch. See <i>Vicia</i> sp.	
<i>Vicia</i> sp., food plant of <i>Agromyza pusilla</i> .....	64
<i>Vigna unguiculata</i> , food plant of <i>Agromyza pusilla</i> .....	64
injury by <i>Agromyza pusilla</i> .....	74
Volck, W. H., and Ballard, W. S. (paper), Winter Spraying with Solutions of Nitrate of Soda.....	437-444
Watermelon. See <i>Citrullus vulgaris</i> .	
Webster, F. M., and Parks, T. H. (paper), Serpentine Leaf-Miner.....	59-88
Weevil, cotton boll, occurrence in Arizona.....	89-98
of <i>Solanum tuberosum</i> , from Andean South America.....	347-352
Wheat. See <i>Triticum sativum</i> .	
Winter Spraying with Solutions of Nitrate of Soda (paper).....	437-444
Wood, creosote penetration of affected by tyloses.....	464-467
durability of, affected by tyloses.....	462-464
hard, tyloses in.....	451
soft, tyloses in.....	458-461
water-logging of affected by tyloses.....	467-468
Yoder, P. A., and LeClerc, J. A. (paper), Environmental Influences on the Physical and Chemical Characteristics of Wheat.....	275-292
<i>Zagrammosoma multilineata</i> , parasite of <i>Agromyza pusilla</i> .....	81
<i>Zygadenus paniculatus</i> , in Tooele Valley, Utah.....	378